

AD _____

Award Number: DAMD17-01-1-0699

TITLE: XIth International Symposium on Cholinergic Mechanisms -
Function and Dysfunction

PRINCIPAL INVESTIGATOR: Israel Silman, Ph.D.

CONTRACTING ORGANIZATION: The Weizmann Institute of Science
Rehovot 76100 Israel

REPORT DATE: July 2002

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20030214 174

REPORT DOCUMENTATION PAGE*Form Approved*
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2002	3. REPORT TYPE AND DATES COVERED Final Proceedings (1 May 01 - 30 Nov 01)	
4. TITLE AND SUBTITLE XIth International Symposium on Cholinergic Mechanisms - Function and Dysfunction			5. FUNDING NUMBERS DAMD17-01-1-0699	
6. AUTHOR(S) : Israel Silman, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Weizmann Institute of Science Rehovot 76100 Israel			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Original contains color plates: All DTIC reproductions will be in black and white.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) none provided				
14. SUBJECT TERMS: cholinergic mechanisms			15. NUMBER OF PAGES 108	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

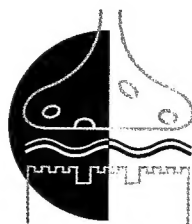


XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY



St. Moritz, Switzerland, May 5 - 9, 2002

**PROGRAM
AND
ABSTRACTS**



XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
**2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY**

St. Moritz, Switzerland
May 5 - 9, 2002



PROGRAM

ORGANIZED BY

KENES *International*
CARES FOR YOUR ORGANIZATION

GLOBAL CONGRESS
ORGANIZERS AND ASSOCIATION
MANAGEMENT SERVICES

P.O. Box 50006, Tel Aviv 61500, Israel
Tel: 972 3 5140018/9

Fax: 972 3 5172484/5140077

E-mail: cholinergic@kenes.com

Website: www.kenes.com/cholinergic



TABLE OF CONTENTS

	Page
Committees	3
Acknowledgements	4
General Information	6
Social Events	8
Accompanying Persons' Program	8
Scientific Program	
Monday, May 6, 2002 – Opening	11
Tuesday, May 7, 2002	13
Wednesday, May 8, 2002	15
Thursday, May 9, 2002	17
Posters	
Monday, May 6, 2002	21
Tuesday, May 7, 2002	24
Wednesday, May 8, 2002	27
Thursday, May 9, 2002	30
Abstracts	33
Poster Abstracts	
Index	





ORGANIZING COMMITTEE

L. Anglister (Jerusalem)
A. Fisher (Ness-Ziona)
D.M. Michaelson (Tel-Aviv)
I. Silman (Rehovot)
H. Soreq (Jerusalem)

INTERNATIONAL ADVISORY BOARD

E.X. Albuquerque (Baltimore)
J.-P. Changeux (Paris)
C. Cuello (Montreal)
B.P. Doctor (Washington DC)
S. Froehner (Seattle)
E. Giacobini (Geneva)
I. Hanin (Maywood)
F. Hucho (Berlin)
N.C. Inestrosa (Santiago)
A. Karczmar (Hines)
P. Kasa (Szeged)
K. Löffelholz (Mainz)
J. Massoulié (Paris)
A. Ménez (Saclay)
Y. Mizuno (Tokyo)
A. Nordberg (Stockholm)
G. Pepeu (Florence)
E.K. Perry (Newcastle upon Tyne)
M.M. Salpeter (Ithaca)
X.C. Tang (Shanghai)
P. Taylor (La Jolla)
S. Tucek (Prague)
V.P. Whittaker (Cambridge)

ACKNOWLEDGEMENTS

The Organizing Committee wishes to express its gratitude to the following, for their generous support:

MAJOR SPONSORS

 **Research Directorate – General**
European Commission Research Directorate – General

Misrahi Foundation



US Army Medical Research Acquisition Activity

SPONSORS



A F M
Association Française
contre les Myopathies

Association Française contre les Myopathies



Eli Lilly and Company

TECHNION Israel Institute of Technology

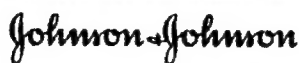
Eve Topf and USA National Parkinson Foundation Centers of Excellence for
Neurodegenerative Diseases, Israel Institute of Technology

INSTITUTE FOR THE STUDY OF AGING

Institute for the Study of Aging, New York



International Society for Neurochemistry



Johnson & Johnson

CONTRIBUTORS



COMMISSARIAT À L'ÉNERGIE ATOMIQUE

Commissariat à l'Energie Atomique DSV/DIEP



EMBO European Molecular Biology Organization



International Union of Biochemistry and Molecular Biology



NOVARTIS

Novartis Pharma AG



Promega

Promega Corporation



Teva Pharmaceuticals



The Dr Josef Cohn Minerva Center for Biomembrane Research, Weizmann Institute



The Harry Stern National Center for Research
of Alzheimer's Disease and Relevant Disorders,
The National Institute of Psychobiology in Israel

The Helen and Milton A Kimmelman Center for
Biomolecular Structure and Assembly, Weizmann Institute

The Joseph and Ceil Mazer Center for Structural Biology, Weizmann Institute



אוניברסיטת תל-אביב
TEL AVIV UNIVERSITY

The Miriam Lebach Chair in Molecular Neurodegeneration, Tel-Aviv University

The Nella and Leon Benozio Center for Neurosciences, Weizmann Institute



GENERAL INFORMATION (Alphabetical)

ACCOMMODATION

Kenes Tours, the official Symposium travel agent, will operate a hospitality desk during the Symposium.

BADGE

On registering you will receive your Symposium kit in which you will find your name badge. You are kindly requested to wear your badge during all sessions and events.

LANGUAGE

The official language of the Symposium is English.

LIST OF PARTICIPANTS

A list of participants who have registered prior to the Symposium is displayed on the bulletin board. Please amend/add your name and address to the list.

LOCATION

Laudinella Hotel, Address: CH-7500, St. Moritz, Switzerland
Tel: +41 81 836 0000; Fax: +41 81 836 0001
E-mail: info@laudinella.ch
Website: www.Laudinella.ch

LUNCHES

An organized lunch is arranged for all participants and accompanying persons staying at the Laudinella hotel, on Monday, Tuesday, Wednesday and Thursday. Participants staying at the Hotel Loffler will also receive lunch vouchers for the Laudinella hotel

MAIL / MESSAGES / MEDICAL ASSISTANCE / LOST & FOUND

Please apply to the Information Desk.

ORGANIZERS AND SECRETARIAT

KENES *International*
CARES FOR YOUR ORGANIZATION

Global Congress Organizers
and Association Management Services
P.O. Box 50006, Tel Aviv 61500, Israel
Tel: +972 3 5140018/9,
Fax: +972 3 5172484 or 5140077
E-mail: cholinergic@kenes.com
Website: www.kenes.com/cholinergic



POSTERS

Posters will be on display on Monday, Tuesday, Wednesday and Thursday and will change every day. Please check your poster scheduling in the program. Your poster should be displayed as per the Board No. in the Program. Posters are to be mounted from 07:45-08:30 on the day your poster is scheduled. Please remove your poster at the conclusion of sessions on your assigned day. The Organizing Committee will not be responsible for posters which are not removed on time. Poster presenters are requested to be present at their poster board during coffee breaks.

REGISTRATION

REGISTRATION/INFORMATION/SECRETARIAT DESKS

Registration desks will be situated at the Laudinella Hotel as follows:
Sunday, May 5 from 16:00 – 20:00.

The desks will reopen on Monday, May 6 at 07:30 and will stay open throughout the sessions on Tuesday, Wednesday and Thursday.

SPEAKERS READY ROOM (Oral Presentations)

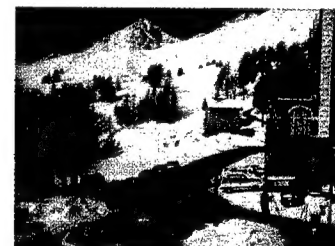
A speakers' ready room will be provided for speakers and will be available every day 30 minutes prior to sessions.

Audiovisual Equipment

1. Overhead projection (projection of transparencies). If using transparencies, please inform the technician in the Speakers' Ready Room at least 30 minutes before the start of your session.
2. Slide projection (projection of 35mm slides). If using slides, you are requested to load them in a slides' tray and check them in the Speakers' Ready Room at least 30 minutes prior to the start of your session.
3. If using a Powerpoint (or any other computer) presentation, please note you need to bring it on a floppy disk (3.5 "/1.44 MB) or on a CD (no ZIP disk !!) and load it on the computer in the session hall, at least 30 minutes before the start of the session (during breaks).

You may supply your own laptop computer **as a back-up**. If using a Macintosh laptop computer, please confirm that it has a VGA socket for external signal and come to check it in the session hall, at least 30 minutes before the start of the session (during breaks).

4. If using video, please ensure that it is VHS multi-system format. Please check it in the session hall, at least 30 minutes before the start of the session (during breaks).





SOCIAL EVENTS

Sunday, May 5, 2002

19:30 Get Together Reception

(included in the registration fees of participants and accompanying persons).

Wednesday, May 8, 2002

21:00 Farewell Dinner

(included in the registration fees of participants and accompanying persons)

ACCOMPANYING PERSONS' PROGRAM

All registered accompanying persons are invited to the Get Together Reception on Sunday and the Farewell Dinner on Wednesday, as well as to the following half-day tour:

Tuesday, May 7, 2002

PONTRESINA

Enjoy a scenic drive from St. Moritz to Pontresina where you will visit the interesting Alpine Museum of mountaineering, hiking and the history of alpinism.

Afterwards, take a leisurely walk around this typically swiss and picturesque village whilst enjoying the breathtaking surrounding natural scenery. Return to St. Moritz will be by horse-drawn sleigh - promised to be an unforgettable experience!

Departure for the tour from the hotel lobby at 09:00 and approximate time of return is 13:00.



XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY



**SCIENTIFIC
PROGRAM**



**This Page Intentionally
Left Blank**

MONDAY, MAY 6, 2002

08:00 – 08:30

WELCOME AND GREETINGS

08:30 – 09:20

PLENARY SESSION 1: HEILBRONN LECTURE

Chairperson: **V.P. Whittaker, UK**

08:30 ACETYLCHOLINE RECEPTORS: PROBING FUNCTIONALLY SIGNIFICANT STRUCTURAL CHANGES WITH SITE-DIRECTED REACTIONS
A. Karlin, USA

09:20 - 10:40

SESSION 1

Chairperson: **F.J. Barrantes, Argentina**

09:20 THE CRYSTAL STRUCTURE OF MOLLUSCAN AChBP REVEALS THE LIGAND BINDING DOMAIN OF THE NICOTINIC ACETYLCHOLINE RECEPTOR
K. Brejc, W.J. van Dijk, R.V. Klaassen, M. Schuurmans, J. van der Oost, A.B. Smit, T.K. Sixma, The Netherlands

09:40 THE BINDING SITE OF THE ACETYLCHOLINE RECEPTOR: FROM SYNTHETIC PEPTIDES TO SOLUTION AND CRYSTAL STRUCTURE
S. Fuchs, R. Kasher, M. Balass, T. Scherf, M. Harel, A. Nicolas, M. Fridkin, J.L. Sussman, E. Katchalski-Katzir, Israel

10:00 STRUCTURAL STUDIES ON THE NICOTINIC ACETYLCHOLINE RECEPTOR
Y. Paas, France

10:20 MOLECULAR BASIS OF THE SELECTIVITY OF NEUROTOXINS TOWARDS nAChR SUBTYPES
D. Servent, C. Gaillard, B. Gilquin, S. Antil-Delbeke, P.J. Corringer, J.P. Changeux, A. Menez, France

10:40 *Coffee Break and Poster Viewing*

11:10 - 12:50

SESSION 2

Chairperson: **D. Bertrand, Switzerland**

11:10 ALLOSTERISM OF THE NICOTINIC ACETYLCHOLINE RECEPTOR
F. Hucho, G.M. Bixel, M. Krauss, Germany

11:30 STRUCTURE AND DYNAMICS OF ACETYLCHOLINE RECEPTOR AND ITS LIPID MICROENVIRONMENT: MOLECULE TO CELL
S. Antollini, J. Baier, M. Blanton, I. Bonini, B. De los Santos, M.C. Gallegos, I. Garbus, M.F. Pediconi, M. Prieto, A.M. Roccamo, J. Wenz, F.J. Barrantes, Argentina

11:50 SITE-DIRECTED REACTIVE PROBES FOR STRUCTURAL AND FUNCTIONAL INVESTIGATION OF CHOLINERGIC PROTEINS
F. Kotzyba-Hibert, S. Ludwig, C. Che, T. Grutter, A. Specht, A. Mourot, M. Goeldner, France

12:10 NMR STRUCTURE OF ALPHA-BUNGAROTOXIN IN COMPLEX WITH AN AChR ALPHA-SUBUNIT PEPTIDE REVEALS THE BASIS FOR SPECIES SPECIFIC RESISTANCE TO THE TOXIN AND HOW ALPHA-NEUROTOXINS INHIBIT ACETYLCHOLINE BINDING TO THE RECEPTOR
A.O. Samson, E. Rodriguez, T. Scherf, J. Anglister, Israel

12:30 ACTIVATION INHIBITION AND UPREGULATION OF THE HUMAN NEURONAL NICOTINIC ALPHA4/BETA2 RECEPTOR BY A PARTIAL AGONIST
V. Itier, D.C. Bertrand, Switzerland

12:50 *Lunch Break and Poster Viewing*

MONDAY, MAY 6, 2002

14:45 - 16:25

SESSION 3

Chairperson: **S.C. Froehner, USA**

- 14:45 ORGANIZATION OF CYTOSKELETON OF MUSCLE FIBERS BY MUSCLE ACTIVITY AND AGRIN
G. Bezakova, **T.L Lomo**, *Switzerland, Norway*
- 15:05 THE ROLE OF PSY1 NUCLEOTIDE RECEPTOR IN THE FORMATION OF NEUROMUSCULAR JUNCTIONS
K.W.K. Tsim, E.A. Barnard, *Hong Kong, UK*
- 15:25 THE DYSTROPHIN COMPLEX - A SCAFFOLD FOR SIGNALING PROTEINS AT SYNAPSES
M.E. Adams, **S.C. Froehner**, *USA*
- 15:45 AGRIN BLOCKADE IMPAIRS LATE BUT NOT INITIAL STAGES OF FUNCTIONAL INNERVATION OF HUMAN MUSCLE IN VITRO
T. Mars, K. Mis, M.P. King, A.F. Miranda, **Z. Grubic**, *Slovenia, USA*
- 16:05 ORGANIZING ACETYLCHOLINESTERASE MOLECULES AT THE NEUROMUSCULAR SYNAPSE
R.L. Rotundo, S.G. Rossi, J.M. Quintero, L.M. Kimbell, *USA*
- 16:25 *Coffee Break and Poster Viewing*
-

17:00 - 18:20

SESSION 4

Chairperson: **J. Massoulié, France**

- 17:00 THE FUNCTIONAL HETERO-OLIGOMERIC FORMS OF CHOLINESTERASES
J. Massoulié, *France*
- 17:20 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL EVENTS CONTROLLING EXPRESSION OF ACETYLCHOLINESTERASE IN DEVELOPING AND ADULT MUSCLES
B.J. Jasmin, L.M. Angus, G. Belanger, J. Deschenes, F. Nassrallah, *Canada*
- 17:40 DENSITY AND LOCALIZATION OF ACETYLCHOLINESTERASE IN VERTEBRATE NEUROMUSCULAR JUNCTIONS
L. Anglister, *Israel*
- 18:00 FOUR ACETYLCHOLINESTERASE GENES IN THE NEMATODE CAENORHABDITIS ELEGANS
D. Combes, Y. Fedon, **J-P. Toutant**, M. Arpagaus, *France*
-

20:30 - 21:50

SESSION 5

Chairperson: **H. Soreq, Israel**

- 20:30 THE MOLECULAR NEUROBIOLOGY OF ACETYLCHOLINESTERASE VARIANTS: FROM STRESSFUL INSULTS TO ANTISENSE INTERVENTION
H. Soreq, D. Glick, *Israel*
- 20:50 TRANSCRIPTIONAL CONTROL OF THE CHOLINERGIC GENE LOCUS (CGL): A MOSAIC MODEL FOR REGULATION OF THE CHOLINERGIC PHENOTYPE
L.E. Elden, B. Schuetz, M. Goerdes, C. Depboylu, M.K-H. Schafer, E. Weihe, *USA, Germany*
- 21:10 REGULATION OF CHOLINERGIC GENE EXPRESSION BY NRSF/REST
M. Shimojo, **L.B. Hersh**, *USA*
- 21:30 NEUROTRANSMITTER PHENOTYPE SWITCH IN DEVELOPING NEURONS – GENOMIC AND PROTEOMIC APPROACHES
M. Linial, Y. Bledi, Y. Bogoch, *Israel*

TUESDAY, MAY 7, 2002

08:30 - 09:20

PLENARY SESSION 2: COUTEAUX LECTURE

Chairperson: **S. Tsuji**, *France*

08:30 WANDERINGS IN AND ABOUT ACTIVE SITE GORGES AND SUBUNIT INTERFACES
P. Taylor, B. Molles, J. Shi, S. Camp, Z. Radic, *USA*

09:20 - 10:40

SESSION 6

Chairperson: **J.M. Gonzalez-Ros**, *Spain*

09:20 REGULATION OF NEUROTRANSMITTER RELEASE: CALCIUM AND ION CHANNELS
R. Rahamimoff, S. Kachalsky, N. Melamed-Book, I. Kaiserman, R. Ahdut, A. Fendyur, A. Raveh, L. Shani, P. Blank, J. Coorsen, J. Zimmerberg, *Israel, USA*

09:40 MACROMOLECULAR ARCHITECTURE OF ACTIVE ZONE MATERIAL AND ITS ROLE IN SYNAPTIC TRANSMISSION
U.J. McMahan, *USA*

10:00 PERSISTENT AND TRANSIENT INHIBITION OF ACETYLCHOLINE RELEASE FROM MOTOR TERMINALS BY BOTULINUM TOXIN A AND E ARE DUE TO THEIR CLEAVAGE PRODUCTS (SNAP-25 1-197 AND 1-180) HAVING DIFFERENT LIFE-TIMES
J.O. Dolly, G.O. Lisk, F.A. Meunier, N. Mohammed, P.G.P. Foran, *UK*

10:20 MEDIATOPHORE NO LONGER AN ARTEFACT
M. Israel, Y. Dunant, *Switzerland, France*

10:40 *Coffee Break and Poster Viewing*

11:10 - 12:50

SESSION 7

Chairperson: **I. Parnas**, *Israel*

11:10 MUSCARINIC PRESYNAPTIC RECEPTORS ARE INVOLVED IN THE CONTROL OF THE TIME COURSE OF NEUROTRANSMITTER RELEASE IN FROG AND MOUSE NEUROMUSCULAR JUNCTIONS
I. Parnas, I. Slutsky, **H. Parnas**, *Israel*

11:30 STRUCTURAL AND FUNCTIONAL CONSERVATION OF SNARE COMPLEXES
D. Fasshauer, W. Antonin, M. Margittai, S. Pabst, R. Jahn, *Germany*

11:50 GENETIC DISSECTION OF SYNAPTIC FUNCTION IN DROSOPHILA
T.L. Schwarz, *USA*

12:10 GENETIC REGULATION OF CHOLINERGIC NEUROTRANSMITTER PHENOTYPES
P.M. Salvaterra, M-H. Lee, S. Song, *USA*

12:30 SIGNALING PATHWAYS THAT REGULATE THE CHOLINERGIC GENE LOCUS EXPRESSION
B. Berse, I. Lopez-Coviella, T. Mellott, B.E. Slack, M.T. Follettie, R.S. Thies, L. Li, J.K. Blusztajn, *USA*

12:50 *Lunch Break and Poster Viewing*

TUESDAY, MAY 7, 2002

14:45 - 16:25

SESSION 8

Chairperson: **P. Marchot**, *France*

- 14:45 COMPARATIVE STRUCTURAL STUDIES ON CONJUGATES OF *TORPEDO CALIFORNICA* AND HUMAN ACETYLCHOLINESTERASES WITH ORGANOPHOSPHATE NERVE AGENTS
J.L. Sussman, C.B. Millard, G. Koellner, G. Kryger, M. Harel, H. Greenblatt, H. Dvir, P. Bar-On, V. Neduva, K. Giles, A. Ordentlich, Y. Segall, N. Ariel, D. Barak, B. Velan, A. Shafferman, L. Toker, I. Silman, *Israel*
- 15:05 CRYSTAL STRUCTURE OF RECOMBINANT HUMAN BUTYRYLCHOLINESTERASE: NEW INSIGHTS INTO THE CATALYTIC MECHANISMS OF CHOLINESTERASES
Y. Nicolet, F. Nachon, P. Masson, O. Lockridge, J-C. Fontecilla-Camps, *France, USA*
- 15:25 INTRINSIC TRYPTOPHAN FLUORESCENCE OF CHOLINESTERASES: DIRECT, NON-PERTURBING MONITORING OF ENZYME-LIGAND INTERACTIONS
Z. Radic, E. Kim, P. Taylor, *USA*
- 15:45 SURPRISING FINDINGS FROM THE FUNCTIONAL ANALYSIS OF HUMAN ACETYLCHOLINESTERASE ADDUCTS OF ALZHEIMER'S DISEASE DRUGS
A. Ordentlich, C. Kronman, D. Barak, N. Ariel, D. Kaplan, B. Velan, A. Shafferman, *Israel*
- 16:05 UNFOLDING AND FOLDING OF *TORPEDO CALIFORNICA* ACETYLCHOLINESTERASE
L. Weiner, C.B. Millard, I. Shin, E. Roth, D. Kreimer, I. Silman, *Israel*
- 16:25 *Coffee Break and Poster Viewing*

17:00 - 18:20

SESSION 9

Chairperson: **P.Y. Masson**, *France*

- 17:00 SCAVENGER PROTECTION AGAINST ORGANOPHOSPHATES BY CHOLINESTERASES
B.P. Doctor, A. Saxena, M.T. Clark, Y. Rosenburg, D.M. Maxwell, D.E. Lenz, Y. Ashani, *USA, Israel*
- 17:20 A COMPLEX ARRAY OF POST-TRANSLATION MODIFICATIONS DETERMINES THE CIRCULATORY LONGEVITY OF ACETYLCHOLINESTERASE IN A HIERARCHIAL MANNER
C. Kronman, T. Chitlaru, A. Ordentlich, B. Velan, **A. Shafferman**, *Israel*
- 17:40 PRESYNAPTIC INHIBITION OF CENTRAL ACETYLCHOLINE RELEASE WITH A1 LIGANDS: PREVENTION OF CHOLINERGIC CRISIS
H.P.M. van Helden, T.J.H. Bueters, B. Groen, M. Danhof, A.P. Ijzerman, *The Netherlands*
- 18:00 POLYURETHANE IMMOBILIZED ENZYMES: OP SENSING AND DECONTAMINATING MATRIXES
R.K. Gordon, B.P. Doctor, S.R. Feaster, A.T. Gunduz, E.D. Clarkson, D.E. Lenz, D.M. Maxwell, T. Cronin, J.P. Skvorak, M.C. Ross, *USA*

WEDNESDAY, MAY 8, 2002

08:30 - 09:20

PLENARY SESSION 3: SALPETER LECTURE

Chairperson: **U.J. McMahan, USA**

08:30 CHEMICAL KINETICS PARAMETERS AND RECEPTOR DEGRADATION RATES AT THE NEUROMUSCULAR JUNCTION
E.E. Salpeter, USA

09:20 - 10:40

SESSION 10

Chairperson: **S. Fuchs, Israel**

09:20 CONGENITAL MYASTHENIC SYNDROMES (CMS): MULTIPLE MOLECULAR TARGETS AT THE NEUROMUSCULAR JUNCTION
A.G. Engel, K. Ohno, S.M. Sine, USA

09:40 ANTIBODIES TO ACETYLCHOLINE RECEPTORS AND MUSK IN MYASTHENIA GRAVIS AND RELATED DISORDERS
A. Vincent, UK

10:00 INHIBITORY EFFECTS OF MUSCARINIC RECEPTOR AUTOANTIBODIES ON PARASYMPATHETIC NEUROTRANSMISSION IN SJOGREN'S SYNDROME
S.A. Waterman, S. Lester, T.P. Gordon, M. Rischmueller, Australia

10:20 IMMUNOTHERAPY OF MYASTHENIA GRAVIS: ANTIGEN-SPECIFIC MUCOSAL TOLERANCE AND ANTAGONISTS OF KEY CYTOKINES AND COSTIMULATORY FACTORS
M.C. Souroujon, S-H. Im, S. Fuchs, Israel

10:40 THE ALPHA7 NACHR L250T MUTATION IN MICE: A MODEL FOR EPILEPSY IN MEN
A. Orr-Urtreger, R.A. Sack, M. Kedmi, A. Harmelin, Z. Gil, Israel

Coffee Break and Poster Viewing

11:10 - 12:50

SESSION 11

Chairperson: **N.C. Inestrosa, Chile**

11:10 NEURODEGENERATIVE PROCESSES IN ALZHEIMER'S DISEASE
N.C. Inestrosa, G.V. De Ferrari, J.L. Garrido, A. Alvarez, M. Bronfman, Chile

11:30 ACETYLCHOLINESTERASE FACILITATES AMYLOID DEPOSITION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE
T. Rees, P. Hammond, S. Younkin, H. Soreq, S. Brimijoin, USA, Israel

11:50 ACETYLCHOLINE HYDROLYSIS AT THE MAMMALIAN SKELETAL NEUROMUSCULAR JUNCTION: MORE THAN ONE ENZYME
E. Krejci, J. Minic, J. Molgo, France

12:10 MOLECULAR AND FUNCTIONAL DIVERSITY IN NICOTINIC ACETYLCHOLINE RECEPTOR GENE FAMILIES OF *C. ELEGANS* AND *D. MELANOGASTER*
D.B. Sattelle, UK

12:30 GENETIC DISSECTION OF AN ACETYLCHOLINE RECEPTOR INVOLVED IN NEURONAL DEGENERATION
M. Treinin, S. Halevi, L. Yassin, Israel

12:50 *Lunch Break and Poster Viewing*

WEDNESDAY, MAY 8, 2002

14:15 - 15:55

SESSION 12

Chairperson: **V.I. Tsetlin, *Russia***

- 14:15 NEURONAL NICOTINIC RECEPTORS, ALLOSTERIC POTENTIATING LIGANDS (apls), AND ENDOGENOUS METABOLITES: IMPLICATIONS FOR TREATMENT OF ALZHEIMER'S DISEASE (AD)
E.X. Albuquerque, M.D. Santos, M. Alkondon, E.K. Moon, A. Maelicke, *USA, Brazil, Germany*
- 14:35 THE RATIONALE FOR USING GALANTAMINE TO TREAT DIFFERENT DEMENTIA TYPES
A. Maelicke, S. Lilienfeld, C. Grantham, *Germany, USA, Belgium*
- 14:55 PREVENTION OF APOPTOSIS BY GALANTAMINE: A NOVEL THERAPEUTIC STRATEGY FOR ALZHEIMER'S DISEASE
M. Garcia-Lopez, *Spain*
- 15:15 PRECLINICAL STUDIES OF GALANTAMINE USING A FORM OF ASSOCIATIVE LEARNING SEVERELY IMPAIRED IN ALZHEIMER'S DISEASE
D. Woodruff-Pak, *USA*
- 15:35 A COMMON AGONIST AND POTENTIATOR FOR ALPHA7 NICOTINIC AND 5-HT₂ SEROTONIN RECEPTORS
R. Zwart, L. Broad, C. Felthouse, K. Pearson, G. McPhie, E. Sher, *UK*
- 15:55 *Coffee Break and Poster Viewing*
-

16:30 - 17:50

SESSION 13

Chairperson: **U.Z. Littauer, *Israel***

- 16:30 MOLECULAR BIOLOGY OF ALZHEIMER'S DISEASE – THERAPEUTIC PERSPECTIVES
R. Nitsch, *Switzerland*
- 16:50 BETA-AMYLOIDS, TAU HYPERPHOSPHORYLATION AND COGNITION ARE BENEFICIALLY AFFECTED BY M1 MUSCARINIC AGONISTS - PERSPECTIVES IN ALZHEIMER'S DISEASE TREATMENT
A. Fisher, Z. Pittel, R. Brandeis, R. Haring, N. Bar-Her, H. Sonogo, I. Marcovitch, N. Natan, N. Maestre-Frances, N. Bons, *Israel, France*
- 17:10 CHOLINERGIC DEFICITS AND NON-COGNITIVE BEHAVIOURAL CHANGES IN PATIENTS WITH DEMENTIA
P. Francis, C.P.L-H. Chen, M.M. Esiri, J. Keene, *UK*
- 17:30 CROSS-TALK BETWEEN APOLIPOPROTEIN E THE AMYLOID PRECURSOR PROTEIN AND BRAIN INFLAMMATION
D.M. Michaelson, S. Meilin, G. Ophir, Y. Ezra, L. Oron, S.M. Beni, E. Shohami, *Israel*

THURSDAY, MAY 9, 2002

08:30 - 09:20

PLENARY SESSION 4: BRZIN LECTURE

Chairperson: **E. Reiner**, *Croatia*

08:30 ACETYLCHOLINESTERASE REGULATION IN SKELETAL MUSCLES
J. Sketelj, N. Crne-Finderle, P. Pregelj, *Slovenia*

09:20 - 10:40

SESSION 14

Chairperson: **U. Drews**, *Germany*

09:20 CHOLINERGIC CORTICAL TERMINATIONS ESTABLISH CLASSICAL SYNAPSES AND UNDERGO AGE-RELATED ATROPHY
A.C. Cuello, P. Turrini, M.A. Casu, T.P. Wong, Y. De Koninck, A. Ribeiro Da Silva, *Canada*

09:40 DEVELOPMENT OF CHOLINERGIC PROJECTIONS TO CORTEX: POSSIBLE ROLE OF NEUROTROPHINS IN TARGET SELECTION.
R. Robertson, J. Yu, *USA*

10:00 ACTIVATION OF THE CHOLINERGIC SYSTEM DURING COGNITIVE PROCESSES
G. Pepeu, M.G. Giovannini, M. Pazzagli, J. Cangiali, M.B. Passani, *Italy*

10:20 PRECLINICAL AND CLINICAL STUDIES ON THE ROLE OF MUSCARINIC RECEPTORS IN THE PHARMACOTHERAPY OF SCHIZOPHRENIA
F.P. Bymaster, A. Shekhar, K.W. Perry, K. Rasmussen, D. McKinzie, C.C. Felder, *USA*

10:40 *Coffee Break and Poster Viewing*

11:10 - 12:50

SESSION 15

Chairperson: **K. Löffelholz**, *Germany*

11:10 CENTRAL CHOLINERGIC NEURONS IN CULTURE: REGULATION OF SURVIVAL AND FUNCTION
M. Segal, N. Landman, V. Greenberger, *Israel*

11:30 HOW IS THE BRAIN SUPPLIED WITH CHOLINE, BUT PROTECTED AGAINST EXCESS CHOLINE?
K. Löffelholz, J. Klein, *Germany*

11:50 MEASURING CEREBRAL ACETYLCHOLINE ESTERASE ACTIVITY IN ALZHEIMER DEMENTIA BY PET FUNCTIONAL PARAMETRIC IMAGING
K. Herholz, G. Zundorf, B. Bauer, S. Weisenbach, W-D. Heiss, *Germany*

12:10 BLOOD-BRAIN BARRIER DISRUPTION IS ASSOCIATED WITH ABNORMAL CORTICAL THETA RHYTHM GENERATION: THE POTENTIAL INVOLVEMENT OF ACETYLCHOLINESTERASE
E. Aviv, I. Shelef, H. Golan, A. Korn, O. Tomkins, L. Pavlovsky, **A. Friedman**, *Israel*

12:30 CONTROL OF ACETYLCHOLINE RELEASE UNDER STIMULATORY CONDITIONS BY ITS BIOSYNTHETIC PRECURSORS; GLUCOSE AND CHOLINE
J. Klein, S. Kopf, K. Loeffelholz, *Germany*

12:50 *Lunch Break and Poster Viewing*

THURSDAY, MAY 9, 2002

14:45 - 16:25

SESSION 16

Chairperson: **A. Enz, Switzerland**

- 14:45 TREATMENT OF DEMENTIA WITH CHOLINESTERASE INHIBITORS
A.D. Korczyn, Israel
- 15:05 CHOLINESTERASE INHIBITORS STABILIZE COGNITIVE DECLINE IN ALZHEIMER'S DISEASE
E. Giacobini, Switzerland
- 15:25 GENDER DIFFERENCES IN THE ACTIONS OF CHOLINESTERASE INHIBITORS
M. Weinstock, R-H. Wang, Israel
- 15:45 AMYLOID PRECURSOR PROTEIN PROCESSING PROPERTIES OF THE NOVEL NEUROPROTECTIVE CHOLINESTERASE MONOAMINE OXIDASE INHIBITOR, TV3326 AND ITS OPTICAL ISOMER, TV3279
M.B.H. Youdim, M. Phalach-Yogev, O. Bar-Am, M. Weinstock, T. Amit, Israel
- 16:05 NOVEL BIFUNCTIONAL COMPOUNDS ELICITING CHOLINERGIC AND ANTI-INFLAMMATORY ACTIVITY FOR THE TREATMENT OF CNS IMPAIRMENTS
G. Amitai, R. Adani, I. Rabinovitz, G. Sod-Moriah, H. Meshulam, Israel
- 16:25 *Coffee Break and Poster Viewing*
-

17:00 - 18:20

SESSION 17

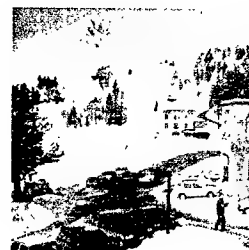
Chairperson: **S. Tucek, Czech Republic**

- 17:00 ROLES OF EXTERNAL LOOPS OF MUSCARINIC RECEPTORS IN INTERACTIONS BETWEEN N-METHYLSCOPOLAMINE AND ALLOSTERIC MODULATORS
A. Krejci, S. Tucek, Czech Republic
- 17:20 STRUCTURE AND ACTIVATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS
E.C. Hulme, Z-L. Lu, M.S. Bee, C.A.M. Curtis, UK
- 17:40 GENERATION AND ANALYSIS OF MUSCARINIC ACETYLCHOLINE RECEPTOR KNOCKOUT MICE
A. Duttaroy, M. Yamada, J. Gomez, W. Zhang, R. Makita, T. Miyakawa, F. Bymaster, C. Felder, C. Deng, J. Wess, USA, Japan
- 18:00 CLOSING REMARKS
A.G. Karczmar, USA

**XIth INTERNATIONAL
SYMPOSIUM ON
CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY**



POSTERS



**This Page Intentionally
Left Blank**

MONDAY, MAY 6, 2002

LIST OF POSTERS 1

- 1 EFFECT OF THYROID HORMONE ON ACETYLCHOLINESTERASE MRNA LEVELS IN THE SLOW SOLEUS AND FAST EDL MUSCLE OF THE RAT
P. Pregelj, J. Sketelj, *Slovenia*
- 2 ACETYLCHOLINESTERASE mRNA EXPRESSION IN RAT SPINAL CORD
K. Mis, E. Davidson, H. Park, M.P. King, T. Mars, Z. Grubic, *Slovenia, USA*
- 3 NEUROMUSCULAR JUNCTION FORMED IN CO-CULTURE WITH EMBRYONIC SPINAL CORD IS ACCOMPANIED BY CO-DIFFERENTIATION OF NEURONAL AND GLIAL CELLS
T. Mars, K.J. Yu, X. Tang, A.F. Miranda, Z. Grubic, F. Cambi, M.P. King, *Slovenia, USA*
- 4 FUNCTIONAL EXPRESSION AND STOICHIOMETRY OF THE NOVEL HUMAN ALPHA9ALPHA10 HETEROMERIC NICOTINIC ACETYLCHOLINE RECEPTOR
C. Grantham, I. Vandenberk, D. Nieuwstraten, P. Groot-Kormelink, L. Van der Helm, J. Yon, A. Kremer, P. Van der Speak, S. Masure, W. Luyten, J. Andrews, *Belgium*
- 5 LARGE SCALE EXPRESSION OF THE EXTRACELLULAR AND CYTOPLASMIC DOMAINS OF THE *DROSOPHILA* ADHESION PROTEIN, GLIOTACTIN
A. Solomon, R. Marcion, D.M. Rees, **E.H. Rydberg**, T. Zeev Ben-Mordechai, S. Botti, I. Silman, J.L. Sussman, V.J. Auld, *Israel, Canada*
- 6 STRUCTURAL REORGANIZATION OF THE ACETYLCHOLINE BINDING SITE OF *TORPEDO* NICOTINIC RECEPTOR REVEALED BY DYNAMIC PHOTOAFFINITY LABELING
T. Grutter, F. Kotzyba-Hibert, S. Bertrand, D. Bertrand, M. Goeldner, *France, Switzerland*
- 7 COEXPRESSION OF ALPHA10 AND ALPHA9 NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT DORSAL ROOT GANGLION NEURONS
K.S. Lips, **U. Pfeil**, R.V. Haberberger, W. Kummer, *Germany*
- 8 MAPPING THE ACETYLCHOLINE BINDING SITES OF *TORPEDO* NICOTINIC RECEPTOR USING PHOTOAFFINITY LABELING: PAST, PRESENT AND FUTURE
F. Kotzyba-Hibert, A. Mourot, T. Grutter, M. Goeldner, *France*
- 9 NFkB REGULATES THE ACTIVITY OF HUMAN ACETYLCHOLINESTERASE PROMOTER IN MUSCLE
R.C.Y. Choi, N.L. Siow, A.W.M. Cheng, D.C.C. Wan, K.W.K. Tsim, *Hong Kong*
- 10 THE INITIAL BINDING OF ACETYLCHOLINESTERASE AND PERLECAN OCCURS INSIDE THE CELL PRIOR TO EXTERNALIZATION
S.G. Rossi, R.L. Rotundo, *USA*
- 11 ROLE OF MDX NERVE AND MUSCLE IN REGULATING NEUROMUSCULAR JUNCTION PROPERTIES: A STUDY USING MUSCLE TRANSPLANTS
A.R. Durrant, M. Szabo, L. Anglister, M.M. Salpeter, *USA, Israel*
- 12 CRYSTAL STRUCTURE OF THE TETRAMERIZATION DOMAIN OF ACETYLCHOLINESTERASE AT 2.3Å RESOLUTION
M. Harel, H. Dvir, S. Bon, W.Q. Liu, C. Garbay, J.L. Sussman, J. Massoulie, I. Silman, *Israel, France*
- 13 SCALING UP OF PRODUCTION, PURIFICATION AND REFOLDING OF A CHIMERIC THREE-FINGERED TOXIN WITH SPECIFICITY FOR ACETYLCHOLINESTERASE
D. Shaya, F. Ducancel, M.H. Le Du, A. Ricciardi, A. Menez, I. Silman, J.L. Sussman, *Israel, France, Uruguay*
- 14 ROLE OF SPONTANEOUS MUTATIONS OF NEURONAL NICOTINIC RECEPTORS IN ADNFLE
D. Bertrand, I. Favre, H. Phillips, S. Bertrand, S.F. Berkovic, J.C. Mulley, *Switzerland, Australia*
- 15 EXPRESSION OF COLQ AT THE NEUROMUSCULAR JUNCTION
C. Legay, *France*

MONDAY, MAY 6, 2002 (continued)

LIST OF POSTERS 1

- 16 INTERACTION OF RECOMBINANT SOLUBLE NEUROLIGINS-1 WITH NEUREXIN-BETA
D. Comoletti, R. Flynn, L. Jennings, R. Hoffman, T. Matsumura, H. Hasegawa, P. Marchot, Y. Bourne, E. Komives, T. Sudhof, P. Taylor, *USA, France*
- 17 CLONING AND CHARACTERIZATION OF ACETYLCHOLINESTERASE GENE IN CHICKEN
X. Zhang, K.W.K. Tsim, **D.C.C. Wan**, *Hong Kong*
- 18 CANDOXIN A NEW SNAKE TOXIN SPECIFIC FOR THE ALPHA7 nAChR
E. Charpantier, S. Nirthanan, P. Gopalakrishnakone, M.C.E. Gwee, H.E. Khoo, L.S. Cheah, R. Manjunatha Kini, D. Bertrand, *Switzerland, Singapore*
- 19 MUSCARINIC RECEPTOR REGULATION OF EVOKED ACETYLCHOLINE RELEASE IS AFFECTED BY ACETYLCHOLINESTERASE INACTIVATION AT THE MOUSE NEUROMUSCULAR JUNCTION
J. Minic, J. Molgo, **E. Krejci**, *France*
- 20 E.COLI EXPRESSED EXTRACELLULAR DOMAIN OF RAT ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR: PHYSICOCHEMICAL AND BINDING PROPERTIES
N.I. Dergousova, E.A. Azeeva, E.V. Kryukova, E.D. Shibanova, I.E. Kasheverov, A.S. Korotina, Y.N. Utkin, **V.I. Tsellin**, *Russia*
- 21 NICOTINIC ACETYLCHOLINE RECEPTOR AND MUSK ARE CLUSTERED IN C2C12 CELLS VIA LIPIDIC RAFTS
F. Stetzkowski-Marden, S. Marchand, J. Cartaud, *France*
- 22 IDENTIFICATION OF SPECIES DIFFERENCES IN THE PHARMACOLOGY OF THE ALPHA-7 NICOTINIC RECEPTOR USING THE ANTAGONIST RADIOLIGAND [3H]-METHYLLYCAONITINE
N. Crawford, K. Finlayson, J. Sharkey, J.S. Kelly, *UK*
- 23 ACETYLCHOLINESTERASE IS REQUIRED FOR NEURONAL AND MUSCULAR DEVELOPMENT IN ZEBRAFISH
M. Behra, X. Cousin, C. Bertrand, J-L. Vonesch, A. Chatonnet, U. Strohle, *France*
- 24 THE 14.3.3 GAMMA PROTEIN IS PART OF THE MUSK SIGNALING COMPLEX AT THE NEUROMUSCULAR JUNCTION
L. Strohlic, A. Cartaud, M. Recouvreur, R. Grailhe, J.P. Changeux, J. Cartaud, *France*
- 25 CONSTRUCTION AND CHARACTERISATION OF A CHIMERIC HUMAN ALPHA 7 NICOTINIC ACETYLCHOLINE / MOUSE 5HT3 RECEPTOR
P.J. Craig, R. Zwart, S. Bose, R.E. Beattie, E.A. Folly, L.R. Johnson, E. Bell, N.M. Evans, S.G. Volsen, E. Sher, N.S. Millar, L.M. Broad, *UK*
- 26 ALPHA-CONOTOXINS PnIA AND A10L-PnIA STABILISE DIFFERENT STATES OF THE CHICK NEURONAL ALPHA 7 ACETYLCHOLINE RECEPTOR
R.C. Hogg, S. Bertrand, P.F. Alewood, D.J. Adams, D.C. Bertrand, *Australia, Switzerland*
- 27 SEGREGATION OF PHOSPHATIDIC ACID-RICH DOMAINS IN RECONSTITUTED ACETYLCHOLINE RECEPTOR MEMBRANES
J.A. Poveda, J.A. Encinar, A.M. Fernandez, M.L. Molina, R. Mateo, **J.M. Gonzalez-Ros**, *Spain*
- 28 RAPSYN ESCORTS THE NICOTINIC ACETYLCHOLINE RECEPTOR ALONG THE EXOCYTIC PATHWAY VIA THE ASSOCIATION WITH LIPID RAFTS
S. Marchand, A. Devillers-Thiery, S. Pons, J-P. Changeux, J. Cartaud, *France*
- 29 IDENTIFICATION AND CHARACTERIZATION OF A DIVERSE FAMILY OF NEUROTOXIN-LIKE PEPTIDES FROM THE SOUTH AMERICAN CORAL SNAKE
T. Kubo, G. Baptista, X. Yang, S. Kobayashi, M. Takeda, A. Prieto-Da-Silva, T. Yamane, *Japan, Brazil*
- 30 MODELS OF THE EXTRACELLULAR DOMAIN OF THE NICOTINIC RECEPTORS AND OF AGONIST AND CA++ BINDING SITES
N. Le Novere, T. Grutter, J-P. Changeux, *France*
- 31 ORIGIN OF ACETYLCHOLINESTERASE IN THE DEVELOPING NEUROMUSCULAR JUNCTION
M. Jevsek, T. Mars, Z. Grubic, *Slovenia*

MONDAY, MAY 6, 2002 (continued)

LIST OF POSTERS 1

- 32 PROBING THE BINDING INTERFACE BETWEEN THE NICOTINIC ACETYLCHOLINE RECEPTOR AND A SHORT ALPHA-NEUROTOXIN THROUGH RECEPTOR-BIOTINYLTUXIN-STREPTAVIDIN TERNARY COMPLEXES
F. Teixeira, A. Menez, **P. Kessler**, *France*
- 33 THE AGONIST BIPHASIC DOSE-RESPONSE CURVE OF THE HUMAN ALPHA4BETA2 RECEPTOR BECOMES MONOPHASIC IN THE PRESENCE OF PKC INHIBITORS OR IN LOW LEVELS OF EXTRACELLULAR CALCIUM IONS
I Bermudez, L.M. Houlihan, *UK*
- 34 NFkB REGULATES THE ACTIVITY OF HUMAN ACETYLCHOLINESTERASE PROMOTER IN MUSCLE
R.C.Y. Choi, N.L. Siow, A.W.M. Cheng, D.C.C. Wan, K.W.K. Tsim, *Hong Kong*
- 35 MOLECULAR CLONING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT GENES FROM THE PEACH-POTATO APHID, *MYZUS PERSICAE*
M. Kirwan, Y. Huang, **M.S. Williamson**, A.L. Devonshire, J.D. Windass, S. Dunbar, S.J. Lansdell, N.S. Millar, *UK*
- 36 NEW ESSENTIAL RESIDUES IN CHOLINESTERASE ACYL POCKET
S.N. Moralev, *Russia*
- 37 VARIABILITY OF SUBSTRATE SPECIFICITY IN CHOLINESTERASES OF VERTEBRATES AND INVERTEBRATES
E.V. Rozengart, **S.N. Moralev**, *Russia*

TUESDAY, MAY 7, 2002

LIST OF POSTERS 2

- 1 2-AMINOPERIMIDINE IS AN EFFECTOR OF CHOLINESTERASES
Y. Shalitin, D. Segal, D. Gur, *Israel*
- 2 PERIPHERAL BINDING OF ETHOPROPAZINE TO HORSE SERUM BUTYRYLCHOLINESTERASE
E. Reiner, G. Sinko, A. Stuglin, V. Simeon-Rudolf, *Croatia*
- 3 SOME CONSIDERATIONS AS TO THE MOLECULAR MECHANISM OF CHOLINESTERASE CATALYSIS
N.B. Brovtsyna, E.V. Rozengart, A.A. Suvorov, S.N. Moralev, *Russia*
- 4 PHOSPHONYLATION OF ACETYLCHOLINESTERASE AND THE PROPENSITY FOR REACTIVATION
ANALYZED BY CHIRALITY AND MUTAGENESIS
Z. Kovarik, Z. Radic, H.A. Berman, P. Taylor, *Croatia, USA*
- 5 THE FIRST TWO NATURALLY OCCURRING ACTIVATORS / REACTIVATORS OF
ACETYLCHOLINESTERASE
R. Gupta, S.S. Thakur, *India*
- 6 KINETICS OF INTERACTION OF ETHOPROPAZINE ENANTIOMERS WITH
BUTYRYLCHOLINESTERASE AND ACETYLCHOLINESTERASE
G. Sinko, Z. Radic, V. Simeon-Rudolf, E. Reiner, P. Taylor, *Croatia, USA*
- 7 REACTIVATION STUDY INDICATES THAT THE ORIENTATIONS OF HI-6 MAY DIFFER IN
REACTIVATING ACETYLCHOLINESTERASE INHIBITED WITH ORGANOPHOSPHATES AND
ORGANOPHOSPHONATES
C. Luo, A. Saxena, H. Leader, Z. Radic, D.M. Maxwell, P. Taylor, B.P. Doctor, *USA*
- 8 KINETIC AND X-RAY CRYSTALLOGRAPHY STUDIES ON THE INTERACTION OF CHOLINESTERASES
WITH THE ANTI-ALZHEIMER DRUG RIVASTIGMINE
P. Bar-On, M. Harel, C.B. Millard, A. Enz, J.L. Sussman, I. Silman, *Israel, USA, Switzerland*
- 9 ATTEMPTS TO ENGINEER AN ENZYME-MIMIC OF BUTYRYLCHOLINESTERASE BY SUBSTITUTION
OF THE SIX DIVERGENT AROMATIC AMINO ACIDS IN THE ACTIVE CENTER OF
ACETYLCHOLINESTERASE
D. Kaplan, A. Ordentlich, D. Barak, N. Ariel, C. Kronman, B. Velan, A. Shafferman, *Israel*
- 10 MALDI-TOF/MS ANALYSIS OF ACETYLCHOLINESTERASE-LIGAND CONJUGATES: A TOOL FOR
RESOLUTION OF MECHANISTIC PATHWAYS
E. Elhanani, A. Ordentlich, O. Dgany, D. Kaplan, Y. Segall, R. Barak, B. Velan,
A. Shafferman, *Israel*
- 11 INFLUENCE OF WATER ON THE FUNCTION OF ACETYLCHOLINESTERASE
R.H. Henchman, K. Tai, T. Shen, J.A. McCammon, *USA*
- 12 THE FLUCTUATING SYNAPSE
K. Kaufmann, *Germany*
- 13 X-RAY STRUCTURE OF TORPEDO ACHE COMPLEXED WITH BIFUNCTIONAL LIGANDS RELATED
TO HUPA: NOVEL DRUGS FOR TREATMENT OF ALZHEIMER'S DISEASE
D.W. Wong, H.M. Greenblatt, D. Shaya, P.R. Carlier, Y.-P. Pang, Y.-F. Han, I. Silman,
J.L. Sussman, *Israel, USA, Hong Kong*
- 14 CRYSTALLIZATION AND DETERMINATION OF THE X-RAY STRUCTURE OF HUMAN AChE
H. Dvir, G. Kryger, J.L. Johnson, T.L. Rosenberry, I. Silman, J.L. Sussman, *Israel, USA*
- 15 3D STRUCTURE OF *TORPEDO CALIFORNICA* ACETYLCHOLINESTERASE COMPLEXED WITH
HUPRINE X
H. Dvir, D.M. Wong, M. Harel, X. Barril, M. Orozco, F.J. Luque, P. Camps, T.L. Rosenberry, I. Silman,
J.L. Sussman, *Israel, Spain, USA*

TUESDAY, MAY 7, 2002 (continued)

LIST OF POSTERS 2

- 16 LIGAND INDUCED CONFORMATIONAL CHANGES IN THE OMEGA LOOP OF ACETYLCHOLINESTERASE REVEALED BY FLUORESCENCE SPECTROSCOPY
J. Shi, Z. Radic, A. Boyd, P. Taylor, *USA*
- 17 *bis*-ACTING GALANTHAMINE DERIVATIVES AS IMPROVED DRUGS IN THE SYMPTOMATIC TREATMENT OF ALZHEIMER'S DISEASE
H.M. Greenblatt, C. Guillou, B. Badet, C. Thal, I. Silman, J.L. Sussman, *Israel, France*
- 18 COMPARISON OF TWO REACTION SCHEMES FOR THE HYDROLYSIS OF ACETYLTHIOCHOLINE BY BUTYRYLCHOLINESTERASE
V. Simeon-Rudolf, G. Sinko, A. Stuglin, J. Stojan, M. Golcnik, E. Reiner, *Croatia, Slovenia*
- 19 QUANTAL ACETYLCHOLINE RELEASE THROUGH MEDIATOPHORE PROTEOLIPID OVER-EXPRESSED IN NEUROBLASTIC CELLS
A. Bloc, J. Falk-Vairant, M. Malo, M. Israel, Y. Dunant, *Switzerland, France*
- 20 STIMULATION OF NICOTINIC RECEPTORS INDIRECTLY INCREASES ACETYLCHOLINE RELEASE IN RAT STRIATUM
V. Dolezal, V. Zemlickova, S. Tucek, *Czech Republic*
- 21 CAPILLARY ZONE ELECTROPHORESIS DETECTS UNWANTED CHOLINESTERASE-BOUND HIDDEN LIGANDS THAT MODULATE ENZYME CONFORMATIONAL STABILITY
D. Rochu, F. Renault, C. Bon, P. Masson, *France*
- 22 CRYSTAL STRUCTURE OF *TORPEDO CALIFORNICA* ACETYLCHOLINESTERASE WITH A NOVEL GALANTHAMINE DERIVATIVE: IMPLICATIONS FOR THE DESIGN OF NEW ANTI-ALZHEIMER DRUGS
M.C. Siotto, C. Bartolucci, **D. Lamba**, *Italy*
- 23 X-RAY STRUCTURE OF SOMAN-AGED HUMAN BUTYRYLCHOLINESTERASE
F. Nachon, Y. Nicolet, P. Masson, J-C. Fontecilla-Camps, O. Lockridge, *France, USA*
- 24 STUDIES ON DYNAMICAL TRANSITIONS IN CHOLINESTERASES
F. Gabel, M. Weik, L. Brochier, D. Fournier, P. Masson, B. P. Doctor, I. Silman, G. Zaccai, *France, USA, Israel*
- 25 TETANIC FADE IS REVEALED BY BLOCKING PRESYNAPTIC NICOTINIC RECEPTORS CONTAINING ALFA4BETA2 AND ALFA3BETA2 SUBUNITS AFTER REDUCING THE SAFETY FACTOR OF NEUROMUSCULAR TRANSMISSION
M. Faria, L. Oliveira, M.A. Timoteo, M.G.B. Lobo, P. Correia-de-Sa, *Portugal*
- 26 MOLECULAR CHARACTERISATION OF ACETYLCHOLINESTERASE FROM THE PEACH-POTATO APHID *MYZUS PERSICAE*(SULZ.)
M.C. Andrews, C.G. Bass, M.S. Williamson, G.D. Moores, *UK*
- 27 PECULIARITIES OF KINETIC BEHAVIOUR OF FISH' S/ABRAMIS BALLERUS/ BLOOD SERUM CHOLINESTERASE
V.D. Tonkopli, *Russia*
- 28 STRUCTURAL INSIGHTS INTO THE INTERACTIONS AT THE ACETYLCHOLINESTERASE PERIPHERAL ANIONIC SITE
Y. Bourne, P. Taylor, H.A. Berman, Z. Radic, **P. Marchot**, *France, USA*
- 29 LETHAL EFFECTS OF HEAD-TO-TAIL 3-ALKYLPYRIDINIUM POLYMERS ISOLATED FROM THE MARINE SPONGE *RANIERA SARAI*: ACHE INHIBITION OR UNSPECIFIC BINDING TO SERUM PROTEINS?
M. Bunc, K. Sepcic, A. Rotter, T. Turk, A. Vidmar, D. Suput, *Slovenia*
- 30 PIPERONYL BUTOXIDE: A SPECIFIC INHIBITOR OF INSECTICIDE RESISTANT ACETYLCHOLINESTERASE
R.V. Gunning, *Australia*
- 31 SIGNIFICANCE OF PARAMETERS BETWEEN VARIOUS KINETIC SCHEMES FOR CHOLINESTERASES
J. Stojan, M. Golcnik, *Slovenia*

TUESDAY, MAY 7, 2002 (continued)

LIST OF POSTERS 2

- 32 STUDIES OF ACETYLCHOLINESTERASE FROM THE PEACH-POTATO APHID, *MYZUS PERSICAE*(SULZ.)
N. Javed, M.S. Williamson, A.L. Devonshire, R.C. Viner, T. Lewis, **G.D. Moores**, *UK*
- 33 EXPLORING THE ACHE GORGE WITH GALANTHAMINE
G. Fels, E. Linnemann, E. Luttmann, C. Pilger, *Germany*
- 34 HYSTERESIS IN BUTYRYLCHOLINESTERASE CATALYSIS: EVIDENCE FOR SUBSTRATE-INDUCED CONVERSION OF THE ENZYME FROM LATENT TO OPERATIVE FORM
P-Y. Masson, M-T. Froment, F. Nachon, L.M. Schopfer, *France, USA*
- 35 A CALCIUM-PROTON ANTIPORT IN CHOLINERGIC AND GLUTAMATERGIC SYNAPTIC VESICLES
M. Cordeiro, V. Bancila, A. Bloc, Y. Dunant, *Switzerland*
- 36 OXACHEIN, A NOVEL POTENT INHIBITOR OF ACETYLCHOLINESTERASE FROM A PLANT-- *OXALIS CORNICULATA* L.
R. Gupta, A. Gupta, *India*
- 37 MECHANISM OF ACETYLCHOLINESTERASE INHIBITION BY FASCICULIN: A 5 NANOSECOND MOLECULAR DYNAMICS SIMULATION
K. Tai, T. Shen, R.H. Henchman, Y. Bourne, P. Marchot, J.A. McCammon, *USA, France*
- 38 PROF. RENE COUTEAUX AND HIS PRESYNAPTIC 'ACTIVE ZONE'
S. Tsuji, *France*

WEDNESDAY, MAY 8, 2002

LIST OF POSTERS 3

- 1 MITOGEN-ACTIVATED PROTEIN KINASE KINASE INHIBITS CILIARY NEUROTROPHIC FACTOR-ACTIVATED CHOLINE ACETYLTRANSFERASE GENE EXPRESSION
T. Mellott, I. Lopez-Coviella, J.K. Blusztajn, **B. Berse**, *USA*
- 2 EFFECTS OF METHOMYL ON SPLEENS AND APOPTOSIS
T. Posayanonda, T. Suramana, N. Nuntharatanapong, O. Lohitnavy, R. Snowden, W. Schwaeble, N. Dusitsin, R. Sindhupak, P. Sinhaseni, *Thailand, UK*
- 3 PLANT CHOLINESTERASE ACTIVITY AS A BIOSENSOR FOR TOXINS IN THE ENVIRONMENT
V.V. Roshchina, *Russia*
- 4 EFFECTS OF CARBAMATE INSECTICIDES ON RAT NEURONAL ALPHA4BETA4 NICOTINIC RECEPTORS AND RAT BRAIN ACETYLCHOLINESTERASE
C.J.G.M. Smulders, T.J.H. Büeters, H.P.M. Vijverberg, *The Netherlands*
- 5 SITE-SPECIFIC ANALYSIS OF GLYCAN STRUCTURES ON PLASMA-DERIVED HUMAN (Hu) AND HORSE (Eq) BUTYRYLCHOLINESTERASES (BChE)
G.E. Garcia, D.R. Moorad-Doctor, O. Lockridge, C.B. Millard, C.A. Broomfield, *USA*
- 6 RECOVERY FROM DESENSITIZATION OF A NEURONAL NICOTINIC RECEPTOR
S. Voytenko, R.J. Lukas, **R. Gruener**, *USA*
- 7 MECHANISM AND STRUCTURAL REQUIREMENTS OF XANOMELINE WASH-RESISTANT BINDING TO M1 MUSCARINIC RECEPTORS
J. Jakubik, E. El-Fakahany, S. Tucek, *Czech Republic, USA*
- 8 IS THE G-PROTEIN-COUPLED M2 MUSCARINIC RECEPTOR A VOLTAGE SENSOR?
O. Tour, N. Dascal, **Y. Ben Chaim**, I. Parnas, H. Parnas, *USA, Israel*
- 9 SOME BASIC RULES GOVERNING OLIGOSACCHARIDE-DEPENDENT CIRCULATORY RESIDENCE OF GLYCOPROTEINS ARE REVEALED BY MALDI-TOF MAPPING OF THE MULTIPLE N-GLYCANS ASSOCIATED WITH RECOMBINANT BOVINE ACETYLCHOLINESTERASE
C. Kronman, T. Chitlaru, N. Seliger, S. Lazar, A. Lazar, L. Zilberstein, B. Velan, A. Shafferman, *Israel*
- 10 EFFECT OF POST-TRANSLATION MODIFICATIONS OF HUMAN ACETYLCHOLINESTERASE ON ITS CIRCULATORY RESIDENCE
T. Chitlaru, **C. Kronman**, S. Lazar, N. Seliger, B. Velan, A. Shafferman, *Israel*
- 11 CHANGES IN NEURONAL CHOLINERGIC RECEPTOR BINDING SITES AT DIFFERENT AGES IN TRANSGENIC MICE OVEREXPRESSING HUMAN ACETYLCHOLINESTERASE
M.M. Svedberg, A-L. Svensson, I. Bednar, A. Nordberg, *Sweden*
- 12 TRANSGENIC OVEREXPRESSION OF READTHROUGH ACETYLCHOLINESTERASE (ACHE-R): DISTRIBUTION OF ACHE-R AND CFOS IN BRAIN IN RELATION TO BEHAVIOR
S. Shoham, O. Cohen, S. Dishon, R. Yirmiyah, E. Kovalev, D. Ginzberg, H. Soreq, *Israel*
- 13 STUDY ON THE MECHANISM OF BLOCKADE OF ACETYLCHOLINE RELEASE BY SNAKE PRESYNAPTIC PLA2 NEUROTOXINS ON NERVE TERMINALS
O. Rossetto, M. Rigoni, P. Caccin, C. Montecucco, *Italy*
- 14 'READTHROUGH' ACETYLCHOLINESTERASE FORMS NEURONAL COMPLEXES WITH PKC BETA II AND ITS WD CARRIER RACK1
E.H. Sklan, K.R. Birikh, S. Shoham, H. Soreq, *Israel*
- 15 THE ROLE OF READTHROUGH ACETYLCHOLINESTERASE IN THE PATHOPHYSIOLOGY OF MYASTHENIA GRAVIS
T. Evron, Y. Hamra, N. Boneva, S. Seidman, T. Brenner, H. Soreq, *Israel*

WEDNESDAY, MAY 8, 2002 (continued)

LIST OF POSTERS 3

- 16 CHRONIC ACETYLCHOLINESTERASE OVEREXPRESSION INDUCES MULTILEVELED ABERRATIONS IN NEUROMUSCULAR PHYSIOLOGY
N. Farchi, H. Soreq, B. Hochner, *Israel*
- 17 EXPRESSION OF THE CHOLINERGIC GENE LOCUS IN THE TRACHEAL EPITHELIUM OF THE RAT
U. Pfeil, L. Eberling, K.S. Lips, R.V. Haberberger, W. Kummer, *Germany*
- 18 MUSCARINIC RECEPTORS AND TRP-CHANNELS IN PRIMARY SENSORY NEURONS OF THE RAT
R. Haberberger, S. Wiegand, M. Kress, *Germany*
- 19 DIVERSE MOLECULAR MECHANISMS UNDERLYING CONGENITAL MYASTHENIC SYNDROMES
R.G. Webster, R. Croxen, S. Brownlow, M. Brydson, S. Haslam, C. Young, C. Slater, J. Newsom-Davis, A. Vincent, D. Beeson, *UK*
- 20 ACETYLCHOLINESTERASE KNOCKOUT MICE HAVE INCREASED SENSITIVITY TO SCOPOLAMINE AND ATROPINE
A. Hrabovska, O. Lockridge, E. Duysen, *USA, Slovak Republic*
- 21 DOWNREGULATION OF MUSCARINIC RECEPTORS IN MICE DEFICIENT IN ACETYLCHOLINESTERASE
B. Li, E.G. Duysen, O. Lockridge, *USA*
- 22 TARGETING OF THE HUMAN VESICULAR ACETYLCHOLINE TRANSPORTER TO CHOLINERGIC SUBDIVISIONS IN TRANSGENIC MICE
B. Schuetz, E. Weihe, L.E. Eiden, *Germany, USA*
- 23 THE DIURNAL ACTIVITY OF ACETYLCHOLINESTERASE INHIBITORS
B.M. Davies, *USA*
- 24 THE MUSCARINIC M1 RECEPTOR AS A THERAPEUTIC TARGET FOR COGNITIVE DEFICITS: PRECLINICAL PHARMACOLOGY AND KNOCKOUT MOUSE STUDIES
C.C. Felder, K.S. Gannon, F.P. Bymaster, A. Porter, D.L. McKinzie, J. Wess, N.M. Nathanson, *UK, USA*
- 25 RESCUE OF THE ACETYLCHOLINESTERASE KNOCKOUT MOUSE BY FEEDING A LIQUID DIET; PHENOTYPE OF THE ADULT ACETYLCHOLINESTERASE DEFICIENT MOUSE
E.G. Duysen, J.A. Stribley, D. Fry, S. Hinrichs, O. Lockridge, *USA*
- 26 ROLE OF MUSCARINIC RECEPTORS IN THE ACTIVATION OF THE SUBICULO-ACCUMBENS PROJECTION
S.N. Mitchell, S. Moss, A. Sharott, *UK*
- 27 BRAIN PENETRATION AND BEHAVIOURAL PROPERTIES OF A POTENT ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST IN THE RAT
N.M. Moore, D.L. McKinzie, **S.N. Mitchell**, M. Keenan, G. Wishart, T.K. Murray, B. Tree, S. Iyengar, J. Hart, D. Shaw, R. Simmons, A. Kalra, C. Miles, J.R. Boot, S.R. Baker, E. Sher, *UK, USA*
- 28 FINE-TUNING MODULATION OF NEURONAL MUSCARINIC M1 (FACILITATORY) AND m2 (INHIBITORY) RECEPTORS ACTIVATION BY ADENOSINE AT THE RAT NEUROMUSCULAR JUNCTION
L. Oliveira, M.A. Timoteo, P. Correia-de-Sa, *Portugal*
- 29 IDENTIFICATION OF SIGNALING PROTEINS DOWNSTREAM OF THE TYROSINE KINASE MUSK IN CLUSTERING OF ACETYLCHOLINE RECEPTORS
R. Willmann, P. Mittaud, C. Fuhrer, *Switzerland*
- 30 DRAMATIC DEPLETION OF CELL SURFACE ACETYLCHOLINE MUSCARINIC RECEPTORS M2R DUE TO LIMITED DELIVERY FROM INTRACYTOSOLIC STORES IN NEURONS OF ACETYLCHOLINESTERASE (ACHE) DEFICIENT MICE.
V. Bernard, C. Brana, I. Liste, O. Lockridge, B. Bloch, *France, USA*
- 31 CHEMICAL MODIFICATION OF RECOMBINANT HUMAN ACETYLCHOLINESTERASE BY POLYETHYLENE GLYCOL GENERATES AN ENZYME WITH EXCEPTIONAL CIRCULATORY LONGEVITY
O. Cohen, C. Kronman, T. Chitlaru, S. Lazar, N. Seliger, D. Kaplan, A. Ordentlich, B. Velan, A. Shafferman, *Israel*

WEDNESDAY, MAY 8, 2002 (continued)

LIST OF POSTERS 3

- 32 HUPERZINE A AND DONEPEZIL ATTENUATE STAUROSPORINE-INDUCED APOPTOSIS IN RAT CORTICAL NEURONS VIA BCL-2 AND BAX REGULATION AND INHIBITION ON CASPASE-3
H.Y. Zhang, X.C. Tang, *China*
- 33 INSECT GROWTH REGULATORS INHIBIT ACETYLCHOLINESTERASE ACTIVITY IN B-BIOTYPE *BEMISIA TABACI* IN AUSTRALIA
E.L.A. Cottage, R.V. Gunning, *Australia*

THURSDAY, MAY 9, 2002

LIST OF POSTERS 4

- 1 BIOCHEMICAL CHARACTERISATION OF MICE TRANSGENIC FOR A MUTATION IN AMYLOID
PRECURSOR PROTEIN (APP) KNOWN TO CAUSE FAMILIAL ALZHEIMER'S DISEASE
P.T. Francis, K.L. Matthews, K.E. Heslop, P.F. Chapman, *UK*
- 2 SYMPATHETIC SUPERIOR CERVICAL GANGLIA (S.C.G.) OF CAT CHOLINERGIC RELAY OF
HYPOTHALAMIC-STIMULATED ORGAN-SPECIFIC VASCULAR CHANGES - RELEVANCY TO
NORMAL AND TO CLINICAL DYSAUTONOMIC FUNCTION
B. Blum, J. Israeli, *Israel*
- 3 CATECHOLAMINE INDUCED CYTOTOXICITY AND ITS PROTECTION, TAURINE AND ANALOGUES:
SOME MORE THOUGHTS
R.C. Gupta, *India*
- 4 THE EFFECT OF TYROSINE ON COGNITIVE FUNCTION IN ANIMAL MODELS FOR ANOREXIA
NERVOSA
D. Ben Shushan, Y. Avraham, S. Hao, S. Mendelson, E.M. Berry, *Israel*
- 5 THE EFFECT OF DIET RESTRICTION, SEPARATION STRESS AND TYROSINE ADMINISTRATION
ON THE CHOLINERGIC SYSTEM IN MICE
S. Hao, **Y. Avraham**, S. Mendelson, E.M. Berry, *Israel*
- 6 EFFECTS OF LITHIUM CHLORIDE ON MEMORY PERFORMANCES OF MICE IN ELEVATED
PLUS-MAZE TEST
P. Yamanturk, L. Eroglu, *Turkey*
- 7 EFFECTS OF 7-NITROINDAZOLE ON MEMORY PERFORMANCES OF RATS TRAINED FOR
THREE-PANEL RUNWAY TASK: HIPPOCAMPAL CHOLINERGIC ENZYME ACTIVITIES
P. Yamanturk, Y. Unlucerci, S. Bekpinar, H. Koyuncuoglu, *Turkey*
- 8 ACETYLCHOLINE AND NO-MEDIATED CGMP SYNTHESIS IN THE RAT BRAIN
W.C.G. Van Staveren, M. Markerink-van Ittersum, H.W.M. Steinbusch, **J. De Vente**,
The Netherlands
- 9 HYDROCORTISONE AFFECTS THE DENSITIES OF CARDIAC MUSCARINIC AND ADRENERGIC
RECEPTORS
J. Myslivecek, J. Ricney, S. Tucek, *Czech Republic*
- 10 EEG EVALUATION OF HUPERZINE A, A REVERSIBLE CHOLINESTERASE INHIBITOR
S.L. Hale, H. Ved, A. Williams, B.P. Doctor, F. Tortella, *USA*
- 11 HUPERZINE A AND CHOLINESTERASE INHIBITORS: GLUTAMATE AND BENZODIAZEPINE
RECEPTOR INTERACTIONS
S.V. Nigam, B.P. Doctor, H.S. Ved, **R.K. Gordon**, *USA*
- 12 CALCIUM MOBILISATION AND CELLULAR CONTRACTION OF EMBRYONIC LENS VESICLE AND
NEURAL TUBE ON MUSCARINIC CHOLINERGIC STIMULATION
U. Drews, M. Oppitz, G. Schriek, *Germany*
- 13 CHOLINERGIC MODULATION OF CHEMOTAXIS IN HUMAN MELANOMA CELLS
A. Boss, S. Noda, M. Sailer, M. Oppitz, U. Drews, *Germany*
- 14 COMBINED ANDROGEN-DONEPEZIL TREATMENT IN POST-STROKE REHABILITATION
J.W. Crayton, L.M. Konopka, **A.G. Karczmar**, *USA*
- 15 LOCALISATION OF THE HIGH-AFFINITY CHOLINE TRANSPORTER-1 IN RAT SKELETAL MUSCLE
AND SPINAL CORD
K.S. Lips, U. Pfeil, **R.V. Haberberger**, W. Kummer, *Germany*
- 16 CHOLINERGIC-GLUTAMATERGIC INTERACTIONS IN HIPPOCAMPAL NEURONS: POSSIBLE ROLE IN
THE NORMAL AND DISEASED HIPPOCAMPUS
L. Pavlovsky, A. Friedman, *Israel*
- 17 HUPERZINE A, A PROMISING ANTI-ALZHEIMER'S AGENT, REDUCES STAUROSPORINE-INDUCED
APOPTOSIS IN NG108-15 CELLS
Y-F. Han, X-Q. Xiao, D-C. Wu, Y. Gao, W-L. Ho, N.T-K. Lee, Y. Fu, K.W.K. Tsim, *Hong Kong*

THURSDAY, MAY 9, 2002 (continued)

LIST OF POSTERS 4

- 18 DOPMINE RELEASE FROM RAT STRIATAL SLICES IN VITRO AND FUNCTIONAL EFFECTS IN 6-OHDA TREATED RATS IN VIVO ARE MEDIATED BY BETA2 CONTAINING NICOTINIC ACETYLCHOLINE RECEPTORS
T.K. Murray, F.A. Jones, D. Steggles, D.R. Dobson, C.P. Dell, **I.A. Pullar**, M.J. O'Neill, *UK*
- 19 THE EFFECTS OF GALANTAMINE IN PATIENTS WITH REFRACTORY SCHIZOPHRENIA RECEIVING RISPERIDONE
J.P. McEvoy, T.B. Allen, *USA*
- 20 CEREBRAL METABOLIC ACTIVATION WITH CHOLINESTERASE INHIBITOR THERAPY IN ALZHEIMER'S DISEASE
M. Mega, I.D. Dinov, M. Manese, J. Felix, S.M. O'Connor, J.L. Cummings, A.W. Toga, *USA*
- 21 THE ROLE OF LIPID PEROXIDATION IN THE MECHANISM OF NEUROTOXICITY OF ORGANOPHOSPHATES
V.D. Tonkopii, *Russia*
- 22 RESCUE OF THE NEURODEGENERATIVE PHENOTYPE IN AD11 ANTI-NGF MICE
S. Capsoni, S. Giannotta, A. Cattaneo, *Italy*
- 23 ACUTE CHOLINERGIC RESCUE OF SYNAPTIC PLASTICITY IN THE NEURODEGENERATING CORTEX OF ANTI-NERVE GROWTH FACTOR MICE
E. Pesavento, **S. Capsoni**, L. Domenici, A. Cattaneo, *Italy*
- 24 THE EFFECT OF NICOTINE ON EXPRESSION OF NICOTINIC RECEPTORS IN THE BRAIN OF PATIENTS WITH ALZHEIMER'S DISEASE
M. Mousavi, E.L. Hellstrom-Lindahl, Z-Z. Guan, K-R. Shan, R. Ravid, A. Nordberg, *Sweden*,
The Netherlands
- 25 A PEPTIDE FROM THE C-TERMINAL OLIGOMERISATION DOMAIN OF HUMAN SYNAPTIC (T-FORM) ACETYLCHOLINESTERASE FORMS CLASSICAL AMYLOID FIBRILS
M.G. Cottingham, M.S. Hollinshead, D.J.T. Vaux, *UK*
- 26 NICOTINE INDUCES GLUTAMATE RELEASE FROM HIPPOCAMPAL MOSSY FIBRES SYNAPTOSOMES
V. Bancila, A. Bloc, Y. Dunant, *Switzerland*
- 27 NICOTINIC CHOLINERGIC ACTIVATION OF MAGNOCELLULAR ENDOCRINE NEURONS OF THE HYPOTHALAMUS
M. Zaninetti, E. Tribollet, D. Bertrand, R. Ogier, **M. Raggenbass**, *Switzerland*
- 28 UNDERSTANDING THE DUAL MODE OF ACTION OF REMINYL(R) USING A VIRTUAL SYNAPTIC CLEFT
M. Lazarewicz, A. Spiros, L. Finkel, R. Carr, **H. Geerts**, *USA*
- 29 ALTERED ACTIVITY OF CHOLINERGIC ENZYMES IN MUSCLES AND BRAIN OF THE OBESE-DIABETIC (OB/OB) MOUSE
M.C. Lintern, L. Cooke, H. Scriven, **M.E. Smith**, *UK*
- 30 EFFECT OF PYRIDOSTIGMINE ADMINISTRATION ON ACETYLCHOLINESTERASE AND CHOLINEACETYLTRANSFERASE ACTIVITY IN THE GUINEA-PIG STRIATUM AND CEREBELLUM
M.E. Smith, M.C. Lintern, C.J. Brewer, J.R. Wetherell, *UK*
- 31 NICOTINIC BETA4 RECEPTOR MEDIATED ACETYLCHOLINE RELEASE FROM RAT INTERPEDUNCULAR NUCLEUS
F.A. Jones, L.R. Johnson, N. Evans, S. Bose, P.J. Craig, S.G. Volsen, I.A. Pullar, *UK*
- 32 CALCIUM CONDUCTANCE AND CHOLINE SENSITIVITY OF SLOW CHANNEL SYNDROME ACETYLCHOLINE RECEPTOR MUTANTS
I. Spreadbury, R. Webster, D. Beeson, A. Vincent, *UK*
- 33 BEYOND THE USUAL SUSPECTS. A CHOLINERGIC ROUTE FOR PANIC ATTACKS
M. Battaglia, A. Ogliari, C. Maffei, *Italy*

THURSDAY, MAY 9, 2002 (continued)

LIST OF POSTERS 4

- 34 SELECTIVE HISTOCHEMICAL STAINING OF PERINEURONAL ACETYLCHOLINESTERASE (AChE) IN THE LIVING ENTERIC NERVOUS SYSTEM (ENS) OF RAT AND GUINEA-PIG
S. Tsuji, R. Nakatomi, H. Tsuchiya, I. Motelica-Heino, K. Hirai, Y. Katayama, K. Ishii, T. Hashikawa, *France, Japan*
- 35 NICOTINIC ACETYLCHOLINE RECEPTOR $\alpha 5$ SUBUNITS MODULATE OXOTREMORINE-INDUCED SALIVATION AND TREMOR
N. Wang, A. Orr-Urtreger, J. Chapman, R. Rabinowitz, A.D. Karczyn, *Israel*
- 36 AUTONOMIC FUNCTION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS $\alpha 5$ SUBUNITS
N. Wang, A. Orr-Urtreger, J. Chapman, R. Rabinowitz, R. Nachman, A.D. Karczyn, *Israel*
- 37 DEFICIENCY OF β NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS CAUSES AUTONOMIC CARDIAC AND INTESTINAL DYSFUNCTIONS
N. Wang, A. Orr-Urtreger, J. Chapman, R. Rabinowitz, R. Nachman, A.D. Karczyn, *Israel*
- 38 ALPHA7 ACETYLCHOLINE RECEPTOR IN SCHIZOPHRENIA: DECREASED mRNA LEVELS IN PERIPHERAL BLOOD LYMPHOCYTES
O. Perl, T. Ilani, R.D. Strous, S. Fuchs, *Israel*
- 39 USE OF THE MORPHING GRAPHICS TECHNIQUE TO VISUALIZE CONFORMATIONAL DIFFERENCES BETWEEN ACHES FROM DIFFERENT SPECIES AND INHIBITOR-INDUCED CONFORMATIONAL CHANGES
T. Zeev-Ben-Mordehai, I. Silman, J.L. Sussman, *Israel*

XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY



ABSTRACTS



IN MEMORIAM EDITH HEILBRONN

Victor P. Whittaker

Wolfson College, Cambridge, UK

Edith Heilbronn died on May 11, 1999. She was the main instigator of the series of International Symposia on Cholinergic Mechanisms of which this is the 11th: this is also the first since her death. The Organizing Committee have therefore felt it appropriate that her contribution to cholinergic mechanisms should be honoured at this meeting.

Edith was born in 1925, the only child of a German-Jewish couple, members of the large Jewish community of Fürth, Northern Bavaria. In 1938 the family sought sanctuary in Sweden, where her father had business connections. She started her career as a technician in the Wenner-Grenn Institute but by attending evening classes she obtained her fil lic from Stockholm University. In 1957 she joined the scientific staff of the Swedish Defence Research Establishment. She rose steadily, becoming chief of an enlarged Biochemistry Section within the Department of Chemistry, meanwhile obtaining her doctorate from Uppsala University. She worked on the O/P nerve gases, then on the isolation of the nicotinic acetylcholine receptor and the induction of an experimental form of myasthenia gravis by injecting it into rabbits.

By now well-known internationally, Edith felt the need to bring together others working in the field of cholinergic function. In February 1970 she organized the first ISCM in Skokloster, an old castle near Uppsala. Few who were present will forget the snowy landscape, the outdoor barbecue, the abundantly flowing glögg and above all the exciting exchange of scientific work between most of the leading groups, all testimony to Edith's flair for organization. In 1979 Edith created a Department of Neurochemistry and Neurotoxicology within the Natural Science Faculty of Stockholm University. The stream of students from home and abroad who passed through the department did much to establish the scientific study of these subjects in Sweden and abroad.

THE FUNCTIONAL ORGANIZATION OF BRAIN NICOTINIC RECEPTORS AT THE AMINOACID LEVEL: ACTIVATION, DESENSITIZATION AND UP-REGULATION

Jean-Pierre Changeux

CNRS URA 2182 "Récepteurs et Cognition", Institut Pasteur, Paris, France

The nicotinic receptor (nAChR) pentamer of 300KD carries all the structural elements which account for channel activation, desensitization, and up-regulation by acetylcholine (Corringer *et al.*, 2000). The data obtained by affinity labeling, site-directed mutagenesis, and X-Ray crystallography (with the snail acetylcholine binding protein by Smit & Sixma groups) (Brejc *et al.*, 2001) about the relevant sites and conformational transitions are reviewed (Grutter & Changeux, 2001). The relative contributions of the 6 distinct loops of the ACh binding site located at the boundary between subunits, together with that of the M2 segment and M1-M2 loop from the ion channel are discussed. Structural elements contributing to desensitization and up-regulation are presented. The data are interpreted in terms of a multi-states allosteric model (Edelstein *et al.*, 1997, Changeux and Edelstein, 1998).

References

- Brejc K., van Dijk W.J., Klaassen R.V., Schuurmans M., van der Oost J., Smit A.B. & Sixma T.K. (2001) *Nature*, **411**:269-276.
Corringer P.J., Le Novère N. & Changeux J.P. (2000) *Annu. Rev. Pharmacol. Sci.*, **40**:431-458.
Grutter T. & Changeux J.P. (2001) *Trends in Biochem. Sci.* **26**:459-463.
Edelstein S., Schaad O. & Changeux J.P. (1997) *Biochemistry*, **36**:13755-13760.
Changeux J.P. & Edelstein S. (1998) *Neuron*, **21**:959-980.

ACETYLCHOLINE RECEPTORS: PROBING FUNCTIONALLY SIGNIFICANT STRUCTURAL CHANGES WITH SITE-DIRECTED REACTIONS

A. Karlin

Center for Molecular Recognition, Columbia University, New York, USA

Both in the ACh binding sites and in the cation-conducting channel of the nicotinic ACh receptor, changes in the reactivity of either native cysteines or of cysteines substituted for native residues have been correlated with changes in functional state. Changes in reaction rates of target cysteines reflect changes in the reagent's path to the target and changes in the local environment of the target. We have dissociated these two kinds of influence on the reaction rates to determine the position of the gate in the channel in both the resting state and the desensitized state and to determine the intrinsic electrostatic potential in the binding site and in the channel. Widespread changes in the reactivity of substituted cysteines in the channel lining reflect an extensive pathway of propagation of the perturbation from the ACh-binding sites to the region of the channel gate.

THE CRYSTAL STRUCTURE OF MOLLUSCAN AChBP REVEALS THE LIGAND BINDING DOMAIN OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

K. Brejc¹, W.J. van Dijk¹, R.V. Klaassen², M. Schuurmans², J. van der Oost², A.B. Smit², T.K. Sixma¹

¹Netherlands Cancer Institute, Amsterdam, ²Vrije Universiteit City, Amsterdam, The Netherlands

We have solved the crystal structure of Acetylcholine binding protein (AChBP), a homolog of a ligand binding domain of the nicotinic acetylcholine receptor (nAChR). AChBP is a glia-produced water-soluble protein from *Lymnaea stagnalis*, which is involved in synaptic modulation. Its homopentamer has sequence and pharmacological similarity to the nAChR ligand-binding domain, but it lacks a transmembrane domain. The nAChRs belong to the superfamily of pentameric ligand gated ion-channels, or Cys-loop receptors, which mediate rapid chemical transmission of signals. These allosteric transmembrane proteins include the nicotinic acetylcholine, the serotonin 5HT₃, GABAA, GABAC and glycine receptors.

The crystal structure shows that in the AChBP protomers have an immunoglobulin-like topology. Ligand-binding sites are located at each of five subunit interfaces and contain residues contributed by biochemically determined 'loops' A to F. The subunit interfaces are highly variable within the ion-channel family, whereas the conserved residues are stabilizing the protomer fold. This AChBP structure is relevant for the development of drugs against e.g. Alzheimer's disease and nicotine addiction.

THE BINDING SITE OF ACETYLCHOLINE RECEPTOR: FROM SYNTHETIC PEPTIDES TO SOLUTION AND CRYSTAL STRUCTURE

S. Fuchs, R. Kasher, M. Balass, T. Scherf, M. Harel, A. Nicolas, M. Fridkin, J.L. Sussman, E. Katchalski-Matzir

Weizmann Institute of Science, Rehovot, Israel

Our group has been employing short synthetic peptides, encompassing sequences from the acetylcholine receptor (AChR) α -subunit for the analysis of the binding site of AChR. A 13-mer peptide mimotope, with similar structural motifs to the AChR binding region, was selected by α -bungarotoxin (α -BTX) from a phage-display peptide library. The solution structure of a complex between this library-lead peptide and α -BTX was solved by NMR spectroscopy, indicating that the bound peptide adopts an almost globular conformation. Based on this NMR study and on structure-function analysis of the AChR binding site, additional 56 peptides, resulting from systematic residue replacement in the lead peptide, one or more replacements at a time, were designed and characterized. Of these, four peptides, designated high affinity peptides (HAPs), homologous to the binding region of AChR, inhibited the binding of α -BTX to AChR with IC_{50} of 2 nM. The solution and crystal structures of complexes of α -BTX with HAP, were solved, demonstrating that the HAP fits snugly to α -BTX and adopts a β -hairpin conformation. The X-ray structures of the bound HAP and the homologous loop of the acetylcholine binding protein (AChBP), are remarkably similar. Their superposition results in a model indicating that α -BTX wraps around the receptor binding-site loop, and in addition, binds tightly at the interface of two of the receptor subunits, where it inserts a finger into the ligand-binding site. Our proposed model explains the strong antagonistic activity of α -BTX, and accommodates many of the biochemical data on the mode of interaction of α -BTX with AChR.

ALLOSTERISM OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

F. Hucho¹, G.M Bixel¹, M. Krauss²

¹Freie Universität, Inst. für Chemie - Biochemie, Berlin, Germany.

²Göttingen, Germany

The nicotinic acetylcholine receptor is an allosteric protein. Agonist binding and channel opening as well as interactions with non-competitive inhibitors (NCIs) are cooperative processes. It has been proposed that the symmetry model (Monod-Wyman-Changeux Model, MWC) describes the receptor's allosterism best. This model postulates: i. a simple axis of symmetry and ii. a preformed equilibrium of functional receptor states, in this case the resting (channel closed), the active (channel open) and the desensitized (channel closed, high agonist affinity) states shifted by ligand binding. Experimentally, it is difficult to discriminate between this and alternative (e.g. the induced fit model) models, because the methods of investigation may affect the equilibrium between states. One prediction of the MWC model is preferential binding of agonists to the activated and desensitized states, and of antagonists to the resting state. We developed a method to 'freeze' the equilibrium by covalent cross-linking and to fix acetylcholine receptors either in the resting or in the desensitized state. Binding studies performed with these fixed-state receptors are not compatible with the MWC model.

MOLECULAR BASIS OF THE SELECTIVITY OF NEUROTOXINS TOWARD nAChR SUBTYPES

D. Servent¹, C. Gaillard¹, B. Gilquin¹, S. Antil-Delbecke¹, P.J. Corringer², J.P. Changeux², A. Ménez¹

¹CEA, Département d'Ingénierie et d'Etudes des Protéines,

Gif-sur-Yvette, ²Laboratoire de Neurobiologie Moléculaire, Institut Pasteur, Paris, France

Snake alpha-neurotoxins block nicotinic acetylcholine receptors (AChRs) from peripheral and neuronal tissues, with high affinity (10^{-9} - 10^{-11} M). alpha-Cbtx, from *Naja kaouthia*, like other long chain neurotoxins, can block muscular-type and alpha7 neuronal receptors. The physiological significance of this property is unclear but the molecular elements associated with this dual activity are identified. Thus a core of toxin residues, at the tip of the toxin central loop, bind to both receptor subtypes, whereas other residues interact to one subtype only. This situation suggests that the binding core binds to receptor residues that are present in both receptor subtypes whereas the subtype-specific toxin residues interact with differential receptor residues. To understand the molecular basis of this selectivity, we performed complementary double mutant cycle experiments between alpha-Cbtx and the neuronal AChR. These data were used to assist a docking calculation between the toxin and a structural model of alpha7 AChR, derived from the recently solved 3D structure of a soluble homopentameric homologue of the extra-cellular domain of AChR. This analysis provides a rational basis for selectivity of protein antagonists that block AChRs.

STRUCTURE AND DYNAMICS OF ACETYLCHOLINE RECEPTOR AND ITS LIPID MICROENVIRONMENT: MOLECULE TO CELL

S. Antollini, J. Baier, M. Blanton, I. Bonini, B. De los Santos, M.C. Gallegos, I. Garbus, M.F. Pediconi, M. Prieto, A.M. Roccamo, J. Wenz, F.J. Barrantes

Instituto de Investigaciones Bioquímicas and UNESCO Chair of Molecular Neurobiology & Biophysics, Bahía Blanca, Argentina, rtfb1@criba.edu.ar

Two approaches to identify the occurrence of lipid sites in the membrane-bound nicotinic acetylcholine receptor (AChR) will be described: a) Förster-type resonance energy transfer (FRET) and fluorescence studies using the probe Laurdan; b) single-channel recordings of AChR mutated in relevant residues of transmembrane (TM) domains. Changes in FRET efficiency induced by fatty acids, phospholipid and cholesterol led to the identification of discrete sites for these lipids on the AChR protein.

Spectroscopy studies of *N*-(1-pyrenyl)maleimide (PM)-labelled intact *Torpedo* AChR protein and TM peptides reconstituted into liposomes have served to explore their topography relative to the bilayer. From spin label quenching of PM-labelled Cys residues in α M1, α M4, γ M1 and γ M4 we could reach the conclusion that they all lie in a shallow position. For M4 segments, this is compatible with a linear α -helical structure, but not so for M1, for which "classical" models locate Cys residues at the centre of the hydrophobic stretch. The TM topography of M1 can be rationalized on the basis of the presence of non-helical structure, and/or of kinks attributable to the occurrence of the evolutionarily conserved proline residues. The latter is a striking feature of M1 in the AChR and in fact in all members of the rapid ligand-gated ion channel superfamily.

The effect of lipids on lipid domain ("raft") formation and AChR stabilization is currently being characterized using fluorescence methods in vitro and in living cells. Cellular studies are aimed at understanding how lipid domains relate to AChR targeting/stabilization to/at the cell surface. Towards this goal, cells expressing AChR are interrogated with fluorescence microscopy in combination with various lipid modification procedures and correlated with Laurdan generalized polarization (GP) studies. Changes in cell-surface fluorescence of alpha-bungarotoxin are observed upon cholesterol and sphingolipid modification, and correlated with modification of AChR targeting to the cell-surface and with changes in the physical state of the plasmalemma in various mammalian AChR-expressing cells.

Supported by FONCYT, UNS, FIRCA (NIH) and Ministerio de Salud Pública.

SITE-DIRECTED REACTIVE PROBES FOR STRUCTURAL AND FUNCTIONAL INVESTIGATION OF CHOLINERGIC PROTEINS

F. Kotzby-Hibert, S. Ludwig, C. Che, T. Grutter, A. Specht, A. Mourou, M. Goeldner

Universite Louis Pasteur Strasbourg, France

The recently solved crystal structure of a glial-derived acetylcholine binding protein (AChBP) [1] will prompt homology modelling of related ligand-gated ion channel proteins and allow the docking of receptor modulators. These models, however, will require biochemical and pharmacological studies for their validation. We have successfully used photoaffinity probes such as [3H] DDF (p-Dimethylamino benzene Diazonium Fluoroborate) and [3H] DCTA (Diaz Cyclohexanoylpropyl Trimethyl Ammonium), to characterize the acetylcholine binding sites on both Torpedo AChR and AChE [2,3] and are presently developing an alternative approach to obtain similar information on recombinant receptors. The method proposes an extension of the SCAM methodology [4] using cysteine-reactive site-directed affinity ligands which react irreversibly with the engineered cysteines [5]. The formation of a specific covalent bond, between selected Cys mutants and high-affinity site-directed labels, demonstrates the specific interaction between the ligand analog and the mutant protein. Such studies will permit accurate ligand receptor interaction studies, by defining precise anchoring points between the receptor and the ligand. Specifically, they will allow the delineation of reliable pharmacophores. The interaction of epibatidine with $\alpha 7$ and $\alpha 4\beta 2$ receptors is being investigated using this approach (Collaboration with D. Bertrand - CMU Geneva). The synthesis and the pharmacological properties of reactive epibatidine derivatives will be described. To gain a dynamic structural insight on functional proteins we also investigated the photoregulation of cholinergic enzyme activities for potential time-resolved crystallographic studies on ChEs [6]. The photoregulation of AChE and BuChE activities was demonstrated using either caged enzyme substrates or by caging directly the catalytic serine of the enzymes.

[1] K. Brejc et al. (2001) Nature 411, 4977-4986.

[2] F. Kotzby-Hibert et al (1995) Angew. Chemie Int. Ed. Engl. 33, 1296-1311.

[3] T. Grutter et al. (2000) Biochemistry 39, 3034-3043.

[4] A. Karlin & M. Akabas (1998) Methods in Enzymol. 293, 123-145.

[5] B. Foucaud et al. (2001) Trends Pharmacol. Sci. 22, 170-173.

[6] L. Peng et al. (1998) Methods in Enzymol. 291, 265-278.

ACTIVATION INHIBITION AND UPREGULATION OF THE HUMAN NEURONAL NICOTINIC $\alpha 4\beta 2$ RECEPTOR BY A PARTIAL AGONIST

Valérie Itier, Daniel C. Bertrand

Department of Physiology, CMU, Geneva, Switzerland

With the expression in cell lines of human neuronal nicotinic receptor it became possible to investigate the properties of these receptors using whole cell recording and very fast drug application. Moreover, these preparations opened the possibilities to better examine the effects of prolonged exposure to a given compound and therefore mimic conditions occurring in the brain. Examination of the activation dose-response curve unveiled that human $\alpha 4\beta 2$ receptors display a high and a low affinity state. Prolonged exposure to nicotine (8 hours) caused a marked displacement of the high versus low affinity ratio, together with an increase of the maximal evoked current. To get a further understanding in this basic mechanism we have examined the effects caused by a partial agonist. Accurate determination of both the activation and inhibition dose-response curves is used to assess the specificity on the high or low affinity states. Short and long term exposures to the partial agonist reveal a differential effect on the receptor function. Comparison of prolonged exposures to an agonist, a competitive antagonist and the partial agonist suggests that activation of the receptor is not required to promote upregulation. These data indicate that chronic exposure of the human nicotinic receptors to selective compounds may cause unexpected increase in the functionality of the cholinergic transmission system.

NMR STRUCTURE OF α -BUNGAROTOXIN IN COMPLEX WITH AN AChR α -SUBUNIT PEPTIDE REVEALS THE BASIS FOR SPECIES SPECIFIC RESISTANCE TO THE TOXIN AND HOW α -NEUROTOXINS INHIBIT ACETYLCHOLINE BINDING TO THE RECEPTOR

Abraham O. Samson¹, Erik Rodriguez¹, Tali Scherf², Jacob Anglister¹

¹Department of Structural Biology and ²Chemical Services, The Weizmann Institute of Science, Rehovot, Israel

The α -subunit of the acetylcholine receptor (α AChR) contains the major binding site for the snake venom derived antagonist α -bungarotoxin (α -BTX). We solved the three-dimensional structure of an α AChR-peptide (residues 182-202) in complex with α -BTX using 2D ¹H-NMR spectroscopy. The bound AChR-peptide adopts a β -hairpin conformation, which associates to the toxin through a novel intermolecular β -sheet, that manifests both hydrophobic and electrostatic interactions resulting in high affinity. This structure correlates the observed changes in toxin binding affinity with mutagenesis studies and with the naturally occurring mutations of α AChR in different animal species and α -subunit types. Based on the structure of a homologous molluscan acetylcholine binding protein, a model of the extracellular domain of the AChR was constructed. Two α -BTXs were docked to this model with the assistance of our NMR data. Lined with aromatic residues, the acetylcholine binding-sites at the interface of α - and $\alpha\delta$ -subunits are occupied by the side-chain of toxin residue Arg-36. This arginine which is conserved amongst all α -neurotoxins sterically prevents acetylcholine from binding to the receptor. These findings coincide with previous mutagenesis studies and illustrate the inhibition mechanism of AChR by α -neurotoxins.

ORGANIZATION OF CYTOSKELETON OF MUSCLE FIBERS BY MUSCLE ACTIVITY AND AGRIN

G. Bezakova¹, T. Lomo²

¹Biozentrum, University of Basel, Switzerland, ²Department of Physiology, University of Oslo, Norway

Contraction and stretch produce mechanical forces in skeletal muscles that are transmitted longitudinally along sarcomeres and transversely across sarcolemmas. For transverse transmission, cytoskeletal F-actin links to dystrophin, which links to transmembrane dystroglycan, and thence to extracellular laminin. Normally, these proteins form transversely oriented stripes over Z-lines (costameres). At neuromuscular junctions (NMJs), cytoskeletal proteins connect to postsynaptic acetylcholine receptors (AChRs) aggregated by nerve-derived agrin. Muscle-derived agrin is almost identical to neural agrin but, owing to differential splicing, lacks a few amino acids needed for AChR aggregation and NMJ formation. Here we search for factors that regulate the organization of cytoskeletal proteins in adult rat soleus muscles using immunocytochemical methods, application of purified nerve- and muscle-derived recombinant agrin, and electrical muscle stimulation.

Denervation caused costameric proteins to become longitudinally oriented. This disorientation was prevented by muscle stimulation or externally applied muscle agrin. Thus, muscle agrin may act in an activity-dependent and autocrine way to organize the cytoskeleton in accordance with the mechanical forces operating in the muscle. Neural agrin caused the appearance of strings of AChR microaggregates that colocalized with dystroglycan, dystrophin, and postsynaptic-specific proteins. In denervated fibers, these strings were longitudinally oriented but switched to a transverse orientation during muscle stimulation or after external application of muscle agrin, suggesting that muscle agrin stabilizes the postsynaptic apparatus by organizing the cytoskeleton to which it is linked. These results provide the first direct evidence for a function of muscle agrin.

THE ROLE OF P2Y1 NUCLEOTIDE RECEPTOR IN THE FORMATION OF NEUROMUSCULAR JUNCTIONS

Karl W.K. Tsim¹, Eric A. Barnard²

¹Departments of Biology and Molecular Neuroscience Center, The Hong Kong University of Science and Technology, Hong Kong, China.

²Department of Pharmacology, University of Cambridge, UK

In vertebrate neuromuscular junctions, adenosine 5-triphosphate (ATP) is stored at the motor nerve terminals and is co-released with acetylcholine during neural stimulation. Several lines of evidence suggest that the synaptic ATP can act as a synapse-organizing factor at the neuromuscular junctions, mediated by metabotropic P2Y1 receptors. P2Y1 receptor mRNA in chicken muscle is very abundant before hatching and again increases in the adult. The P2Y1 receptor protein is shown to be restricted to the neuromuscular junctions and co-localized with AChRs in adult muscle, but not in the chick embryo. In chicks after hatching, this P2Y1 localization develops over about 3 weeks. Denervation or crush of the motor nerve (in chicken or rat) caused decrease in the muscle P2Y1 transcript, which was restored on regeneration. The activation of P2Y1 receptor by adenine nucleotides stimulated the accumulation of inositol phosphates and intracellular Ca²⁺ mobilization in cultured chick myotubes. The receptor activation led to an increase in the expression of transcripts encoding AChR alpha-subunit and AChE. In addition, the expression of P2Y2 and P2Y4 receptors were also restricted at the neuromuscular junctions. These results provide evidence for a novel function of ATP in directing the gene expression of post-synaptic functional effectors.

Acknowledgments:

supported by grants from the Research Grants Council of Hong Kong (HKUST 6099/98M, 6112/00M & 2/99C).

AGRIN BLOCKADE IMPAIRS LATE BUT NOT INITIAL STAGES OF FUNCTIONAL INNERVATION OF HUMAN MUSCLE IN VITRO

T. Mars¹, K. Mis¹, M.P. King², A.F. Miranda³, Z. Grubic¹

¹Institute of Pathophysiology, Medical Faculty, University of Ljubljana, Slovenia. ²Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA, USA. ³Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, USA

Agrin is nerve-derived factor which promotes clustering of components of neuromuscular junctions (NMJ) by the activation of Muscle specific kinase (MuSK). Unlike MuSK deficiency which prevents NMJ formation completely, agrin deficiency permits some degree of synaptogenesis of NMJ. In order to provide more differentiated insight into the role of agrin in the NMJ formation, we followed functional maturation of NMJs in the *in vitro* innervated human muscle under the conditions of agrin blockade. Agrin was blocked by Agr33, a monoclonal antibody demonstrated to efficiently reduce AChR clustering. In the control experiments Agr33 was replaced by Agr86, a monoclonal antibody with little effect on AChR clustering. First and relatively sparse contractions are normally observed between days 7 and 10 of co-culture. Agr33 as well as Agr 86 had no effect in this initial stage of muscle innervation. However, Agr33 -, but not Agr86 - treated co-cultures failed to increase the number of contraction units during the subsequent 5 days when this number significantly increased in the untreated co-cultures. Our results demonstrate that a small population of NMJs reach contraction-competent level relatively early even in the absence of functional agrin, while most of the primarily established neuromuscular contacts need full agrin activity to reach contraction-competent level.

THE DYSTROPHIN COMPLEX – A SCAFFOLD FOR SIGNALING PROTEINS AT SYNAPSES

Marvin E. Adams, Stanley C. Froehner

Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA

One important function of the dystrophin complex in muscle is to link the extracellular matrix (via dystroglycan) to the actin cytoskeleton, thus providing stability to the membrane during contraction. Recent evidence, however, suggests that the dystrophin complex is also a signaling complex. Two classes of dystrophin-associated proteins, the dystrobrevins and the syntrophins, form a scaffold for numerous signaling proteins, including ion channels, kinases and nNOS. Five syntrophins, named α , β 1, β 2, γ 1 and γ 2, are known. Each is a PDZ containing protein. In skeletal muscle of mice lacking α -syntrophin, nNOS and the water channel, aquaporin-4, are absent from the membrane. Furthermore, the neuromuscular junctions are abnormal, with reduced levels of acetylcholine receptor and acetylcholinesterase. The localization of nNOS and aquaporin-4 can be restored by genetic rescue with transgenes expressing α -syntrophin. However, a modified form of α -syntrophin lacking the PDZ domain is not able to restore either nNOS or aquaporin-4 to the membrane. Thus, *in vivo*, both of these proteins require the α -syntrophin PDZ domain. In contrast to muscle, in which aquaporin-4 levels are greatly reduced, the levels in brain are unaffected by the absence of α -syntrophin. However, immunoelectron microscopy reveals that aquaporin-4 is mislocalized and resides mostly on intracellular organelles. Thus, α -syntrophin appears to be involved in regulating the expression, trafficking, and/or localization of certain membrane proteins.

ORGANIZING ACETYLCHOLINESTERASE MOLECULES AT THE NEUROMUSCULAR SYNAPSE

R.L. Rotundo, S.G. Rossi, J.M. Quintero, L.M. Kimbell

Department of Cell Biology and Anatomy, Miami, Florida, USA

The major form of acetylcholinesterase (AChE) at the vertebrate neuromuscular junction (NMJ) is the asymmetric (A12) form consisting of three tetramers of catalytic subunits attached to a collagen-like tail. These molecules are locally synthesized and assembled, and, following externalization, become tightly attached to the synaptic basal lamina. The appearance of AChE on the cell surface is locally controlled by signals generated at the overlying plasma membrane. To study this local control of AChE expression, sealed chambers were used to isolate regions of individual myotubes, and subsequent treatment with agents that increased or blocked membrane depolarization. Blocking membrane depolarization resulted in upregulation of AChE, whereas signals generated in response to membrane depolarization downregulate the enzyme and increased A12 AChE assembly. *In situ* hybridization, RNase protection, and nuclear run on studies suggested that local control occurs at the level of transcription. However, once synthesized, the enzyme is externalized where it accumulates on the extracellular matrix. Several lines of evidence including *in vitro* and *in culture* binding studies, as well as studies using genetically modified mice, indicate that the heparan sulfate proteoglycan perlecan is responsible for localizing AChE on the synaptic basal lamina. Thus, while local regulatory events are responsible for producing sufficient AChE molecules, interactions with extracellular matrix components are responsible for the final localization.

THE FUNCTIONAL HETERO-OLIGOMERIC FORMS OF CHOLINESTERASES

Jean Massoulié

Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS UMR 8544,
Ecole Normale Supérieure, Paris, France

The acetylcholinesterase gene of vertebrates may generate several splice variants which possess the same catalytic domain but differ by their C-terminal regions. However, a single type of catalytic subunit (type T, or AChET) exists in all vertebrate classes, and represents the AChE species which is expressed in the nervous tissue and muscles of mammals. Butyrylcholinesterase (BChE) subunits possess an homologous C-terminal region (T peptide). The T peptide consists of 40-41 residues, which include a cysteine at position -4 of the C-terminus, and a series of seven aromatic residues, including three tryptophans, which are fully conserved in all vertebrates. The T peptide can form an amphiphilic helix with an hydrophobic face covered by these residues. AChE and BChE T subunits can form homo-oligomers, and also associate with anchoring proteins, generating asymmetric forms and membrane-bound tetramers. Our group has cloned a specific collagen, ColQ, which represents the "tail" of asymmetric forms, and more recently a transmembrane protein, PRiMA, which represents the hydrophobic anchor of membrane-bound tetramers. We characterized a proline-rich motif of ColQ, the PRAD ("proline-rich attachment domain"), which is responsible for association with cholinesterases; PRiMA also possesses a proline-rich motif which differs somewhat from the PRAD of ColQ, but clearly serves the same function. We further showed that the interaction relies on the C-terminal T peptide of cholinesterases, which may thus be considered as an autonomous "tryptophan (W) amphiphilic tetramerization" (WAT) domain. Mutagenesis studies showed that the prolines of PRAD and the aromatic residues of WAT play a critical role in this interaction, and that only cysteines located near the C-terminus of the T peptide could form disulfide bonds with the cysteines located at the N-terminal extremity of the PRAD. In collaboration with the groups of I. Silman and J. Sussman and of C. Garbay, we have shown that the PRAD of ColQ forms a complex with four WATs: the four parallel α helices form a cylinder around the PRAD, organized as a polyproline II helix and oriented in the opposite direction; the aromatic side chains are stacked with prolines and the charged residues are located on the outside, and form acidic-basic pairs, within each T peptide. The assembly of PRAD and WATs organizes tetramers of AChET and BChE subunits which are anchored respectively in the basal lamina of neuromuscular junctions, and in the cell membranes, particularly in the central nervous system: ColQ and PRiMA therefore condition the functional localization of cholinesterases.

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL EVENTS CONTROLLING EXPRESSION OF ACETYLCHOLINESTERASE IN DEVELOPING AND ADULT MUSCLES

B.J. Jasmin, L.M. Angus, G. Belanger, J. Deschenes, F. Nassrallah

University of Ottawa, Ottawa, Ontario, Canada

Despite recent progress in our understanding of the biosynthetic events regulating AChE in muscle, our knowledge of the specific molecular mechanisms remains fragmentary. Recently, we began to examine this issue using several experimental systems. Our findings showed that the synaptic accumulation of AChE mRNAs results from a preferential activation of the AChE gene in myonuclei close to the neuromuscular junction. Mutation and deletion studies further demonstrated the critical role of the first intron, particularly that of an intronic N-box motif, in regulating the synaptic expression of the AChE gene. Additional studies revealed that this N-box, along with an adjacent E-box, are also critical for increasing transcription of AChE during early myogenesis. In these studies, we noted that the sustained elevation of AChE mRNAs in myotubes also involves post-transcriptional events. Interestingly, post-transcriptional mechanisms also play a key role in regulating the abundance of AChE transcripts in fast vs slow muscles, and in denervated muscles. Yet, the contribution of transcriptional and post-transcriptional events under these experimental conditions appeared subject to developmental influences. Experiments performed using the rat superior cervical ganglia, indicate that these two types of mechanisms play a similar role in regulating AChE in neurons. Thus, regulation of AChE mRNAs in excitable cells depends on the complex interplay between transcriptional and post-transcriptional mechanisms whose relative importance are influenced by the state of differentiation and maturation of the cholinergic synapses.

ACETYLCHOLINE HYDROLYSIS AT THE MAMMALIAN SKELETAL NEUROMUSCULAR JUNCTION: MORE THAN ONE ENZYME

E. Krejci¹, J. Minic², J. Molgo²

¹UMR8544, ENS-CNRS, Paris, ²UPR9040, CNRS, Gif Sur Yvette, France

Two enzymes hydrolyze acetylcholine (ACh) in mammals, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). These two enzymes share a noteworthy molecular polymorphism. These oligomers result from the expression of two catalytic subunits, each encoded by one gene, AChE and BChE, and of two anchors, ColQ and PRiMA. Each anchor has two functions 1) organization of an enzyme tetramer, 2) targeting of the tetramer to the extracellular domain. ColQ anchors tetramers in the basal lamina and PRiMA anchors tetramers on the cell surface. At the neuromuscular junction (NMJ), ACh actions appear multiple since each of the cells that organize the NMJ are sensitive to ACh: 1) the muscle endplate is activated by quantal ACh release, 2) the presynaptic motor nerve terminals generate fasciculation and back-firing after AChE inhibition and 3) the Schwann cell response to ACh release. To understand this complexity, we started genetic approaches to remove cholinesterases oligomers. In the absence of ColQ, the mice live but are affected by a myasthenia, like humans affected by mutations in ColQ. We have shown that in ColQ^{-/-} mice AChE is absent, but BChE is still present in the telogial domain, probably anchored by PRiMA. To evaluate the functional consequence of the absence of AChE and the presence of BChE, we quantified nerve-evoked muscle contraction at different frequencies of nerve stimulation. The muscle failed to maintain a tetanus during repetitive stimulations (higher than 30 Hz). At 10 Hz of nerve stimulations, the contraction of the ColQ mutant is very similar to the control, but the mutant is very sensitive to BChE inhibition, while the control is not affected. This indicates that BChE controls the level of ACh during repetitive stimulations. However, BChE does not play the same role as AChE because miniature Endplate Potentials (mEPPs) and EPPs due to spontaneous and evoked release are not modified after BChE inhibition. This clearly suggests that the ACh level is controlled differently depending on the localization and/or the nature of the enzyme.

DENSITY AND LOCALIZATION OF ACETYLCHOLINESTERASE IN VERTEBRATE NEUROMUSCULAR JUNCTIONS

Lili Anglister

Department of Anatomy and Cell Biology, Hebrew University,
Hadassah Medical School, Jerusalem, Israel

The proper function of synaptic acetylcholinesterase (AChE) is determined by its concentration and position in the synaptic cleft. The present work describes the use of derivatives of fasciculin (Fas2), a polypeptide anticholinesterase toxin, as probes for determination of AChE densities at cholinergic synapses and for high resolution EM- localization of AChE in synaptic clefts.

Comparative studies of AChE densities and distribution at neuromuscular junctions (nmjs) were done by quantitative EM-autoradiography of muscles labeled with radio-iodinated Fas2. These studies revealed several-fold difference in junctional AChE- site densities in muscles of different vertebrates. Junctional AChE sites/ μm^2 in mouse sternomastoid and lizard intercostal endplates were 4 and 2.5-fold higher than in frog cutaneous pectoris nmjs, respectively. AChE sites were distributed over the primary clefts and the full depth of the junctional folds.

Localization studies with nanogold-conjugates of Fas2 revealed that gold-labeled AChE sites were distributed over the basal lamina in the primary cleft and the postjunctional folds. Quantitative data analysis demonstrates that AChE sites are almost exclusively located on the basal lamina rather than pre- or postsynaptic membranes and are distributed in the primary cleft and full depth of the postjunctional folds. This localization pattern of AChE assures the hydrolysis of ACh bouncing off receptors, and eliminates its unnecessary re-binding.

Supported by the Israel Sci. Found. - Israel Acad. Sci. 180/98.

FOUR ACETYLCHOLINESTERASE GENES IN THE NEMATODE CAENORHABDITIS ELEGANS

D. Combes¹, Y. Fedon¹, J.-P. Toutant¹, M. Arpagaus^{1,2}

¹Différenciation Cellulaire et Croissance, ²Ecologie Microbienne et Interaction Hôte-Pathogène, INRA Montpellier, France

Several genes encode acetylcholinesterase (AChE, EC 3.1.1.7) in nematodes. In *Caenorhabditis elegans* we have cloned and sequenced the four ace genes, studied their genomic organization and their tissue-specific expression using GFP reporter constructs transfected in vivo. Two genes, ace-1 and ace-2, encode two major AChEs with different pharmacological properties and tissue repartition. ace-1 is expressed in muscle cells (1,2) and ace-2 is mainly expressed in neurons (3). One likely hypothesis is that both ace-1 and ace-2 contribute (pre- and post-synaptically) to the neuromuscular AChE explaining that single mutants in ace-1 or ace-2 have no alteration in locomotion. Interestingly, ace-1 has a C-terminus homologous to that of the T variant of vertebrate AChE, whereas ace-2 possesses a C-terminal signal for glypiation as the H variant in vertebrates. ace-3 represents a minor proportion of the total AChE activity in *C. elegans* and is expressed in a few cells (pharyngeal muscle cells and CAN cells). ace-3 is highly resistant to the usual inhibitors of AChE. ace-4 is found a few hundred base pairs upstream of ace-3 on chromosome II. Both genes are organized as an operon and are first transcribed as a bicistronic messenger. However no enzyme corresponding to ace-4 was found. 1. Arpagaus et al., 1994, J Biol Chem 269, 9957-65. 2. Culetto et al., 1999, J Mol Biol 290, 951-66. 3. Combes et al., 2000, J Mol Biol 300, 727-42.

REGULATION OF NEUROTRANSMITTER RELEASE: CALCIUM AND ION CHANNELS

Rami Rahamimoff, Sylvia Kachalsky, Naomi Melamed-Book, Igor Kaiserman, Ronit Ahdut, Anna Fendyur, Adi Ravch, Paul Blank, Jens Coorsen, Joshua Zimmerberg

Department of Physiology and the Bernard Katz Minerva Centre for Cell Biophysics, Hebrew University - Hadassah Medical School, Jerusalem, Israel

Release of transmitter from the cholinergic presynaptic nerve terminals is one of the key processes in neuronal communication. It is a highly controlled process and is regulated by a large number of intrinsic and extrinsic factors. Transmitter release regulation is one of the main determinants of synaptic plasticity. It also serves as an important target for the action of drugs and hormones.

Three aspects of the regulation of transmitter release will be discussed: ion channels in the presynaptic nerve terminal surface membrane, the post-fusion control of transmitter release and the calcium dynamics in presynaptic boutons.

Ion channels at the nerve terminal. The main regulator of quantal transmitter release is the intracellular calcium ion concentration. This in turn is regulated by the calcium ions that enter through the surface membrane and the calcium ions released from intracellular stores. These processes are controlled by several hundred of different ion channel molecules that affect directly and indirectly the calcium flux.

The post-fusion control of transmitter release. The cholinergic synaptic vesicle of Torpedo electromotor neurons contains acetylcholine at a very high concentration. Most of the acetylcholine is bound to an intravesicular ion exchange matrix. Entry of cations into the vesicles can displace the transmitter and facilitate its release. We propose that the non-selective ion channels in the vesicle membrane can thus exert a post fusion regulation of transmitter release.

Calcium dynamics in nerve terminals and sea urchin eggs. Four aspects of calcium dynamics in the presynaptic nerve terminals will be discussed in view of their possible role in transmitter release: calcium oscillations, calcium waves, calcium sparks and calcium noise.

WANDERINGS IN AND ABOUT ACTIVE SITE GORGES AND SUBUNIT INTERFACES

Palmer Taylor, Brian Molles, Jian Shi, Shelley Camp, Zoran Radić,

Department of Pharmacology, University of California-San Diego, La Jolla, CA, USA

A wide variety of natural toxins that inhibit motor activity have evolved from both plant and animal species for purposes of predation or protection from predation. The unique specificities of these toxins permit their use to not only distinguish subtypes of receptors or other targets, but also probe the structures of individual binding sites within a single oligomeric receptor. In addition, these toxins and related ligands provide information on the conformational dynamics of the nAChR and AChE not available in crystal structures. We show here the structural bases for the *Conus magnus* toxin's, ∇ -conotoxin-M1, preferential affinity for the ∇^* -subunit interface over ∇ , and $\nabla(\cdot)$ interfaces; the viper toxin's, Waglerin-I, preference for the ∇ , interface and the krait toxin; *Naja mossambica mossambica* I, preference for the ∇^* and $\nabla(\cdot)$ subunits over ∇ . Distinct structural determinants can be defined indicating that discrete regions, formed from distant portions of the linear sequence form the binding site. In fact, these regions show the appropriate proximity relationships in the recently crystallized soluble acetylcholine binding protein from snail. Through the use of pairwise mutations in the receptor and the respective toxins and thermodynamic mutant cycle analysis, it has been possible to determine proximity relationships of residues on the receptor and toxin, orient the toxin within the binding sites, and delineate individual residue contributions to the binding energetics. A second 3-fingered toxin, fasciculins, is a high affinity inhibitor of AChE. Through cysteine substitution mutagenesis and selective fluorophore conjugation, changes in residue microenvironments, solvent exposure and sequential motion of individual side chains have revealed that fasciculins and small ligand binding induces distinctive allosteric changes in the omega loop (Cys 69-Cys 96) of mouse AChE. Hence these physicochemical parameters provide an essential linkage between molecular dynamics computations and the static crystal structures.

(supported by GM18360 and DAMD 1718014).

MACROMOLECULAR ARCHITECTURE OF ACTIVE ZONE MATERIAL AND ITS ROLE IN SYNAPTIC TRANSMISSION

Uel J. McMahan

Department of Neurobiology, Stanford University School of Medicine, Stanford, CA, USA

The position of active zone material at the nervous system's synapses, next to the synaptic vesicles docked at the presynaptic plasma membrane and to calcium channels within the membrane-- both of which are directly involved in neurotransmitter release during impulse transmission -- has raised questions as to the material's relationship to the vesicles and channels and to its function. My colleagues and I have examined for the first time the intricate arrangement and associations of structural components of this compact, proteinaceous organelle. Our approach was to use electron microscope tomography to generate 3D reconstructions of tissue sections from a model synapse, the frog's neuromuscular junction, and then to apply segmentation and surface rendering schemes for characterizing specific structures within the active zone material. Our findings lead to the hypothesis that the active zone material helps dock synaptic vesicles and anchor calcium channels and that the architecture of the material provides for both a particular spatial relationship and a structural linkage between the vesicles and channels. The structural linkage may well include proteins that mediate the calcium-triggered exocytosis of neurotransmitter by synaptic vesicles during synaptic impulse transmission.

PERSISTENT AND TRANSIENT INHIBITION OF ACETYLCHOLINE RELEASE FROM MOTOR TERMINALS BY BOTULINUM TOXIN A AND E ARE DUE TO THEIR CLEAVAGE PRODUCTS (SNAP-25 1-197 AND 1-180) HAVING DIFFERENT LIFE-TIMES

J.O. Dolly, G.O. Lisk, F.A. Meunier, N. Mohammed, P.G.P. Foran

Department of Biological Sciences, Imperial College, London, UK

Botulinum neurotoxin (BoNT) types A-G target peripheral cholinergic neurons and selectively cleave SNAP-25 (A, C1, E), syntaxin (C1) and synaptobrevin (B, D, F & G) – proteins comprising the synaptic vesicle docking-fusion complex responsible for transmitter release. The toxins exhibit amazing specificities and potencies in causing neuromuscular paralysis but for different periods, with type A having the longest duration (> year in humans), hence, its widespread clinical use for the treatment of dystonias. Durations of neuromuscular paralysis by the toxins in rodents were shown to be A>B>F>E; also, BoNT/E shortened the paralysis time for type A, precluding a persistence of its protease for longer than that of type E, at human and murine endplates. Instead, the BoNT/A-cleaved target (SNAP-25_A i.e. 1-197), which is known to directly inhibit exocytosis, persisted for >40 days at the presynaptic membrane. In contrast, E-cleaved SNAP-25 was removed rapidly and, accordingly, E hastened the removal of SNAP-25_A and induced its translocation from the plasmalemma, allowing resumption of transmitter release. At a therapeutic dose, BoNT/A cleaved only ~ 10% of SNAP-25 near its entry sites before being diluted by diffusion; the SNAP-25_A persists at the active zones and could block acetylcholine release by forming disabled SNARE complexes. Removal of an extra 17 C-ter. residues by E prevents such associations and promotes turnover, culminating in resumption of neuromuscular transmission.

MUSCARINIC PRESYNAPTIC RECEPTORS ARE INVOLVED IN THE CONTROL OF THE TIME COURSE OF NEUROTRANSMITTER RELEASE IN FROG AND MOUSE NEUROMUSCULAR JUNCTIONS

I. Parnas, I. Slutsky, H. Parnas

*Otto Loewi Center for Cellular and Molecular Neurobiology,
The Hebrew University of Jerusalem, Israel*

Neurotransmitter release (amount and kinetics) in fast synapses is considered to be controlled by [Ca²⁺]_i. Recent studies suggest that the action potential plays a direct role in determining the kinetics of release by a mechanism which involves presynaptic autoreceptors. Perfusion of a frog nmj with either the M2/M4 muscarinic antagonist, methoctramine, or with exogenous AChE, prolongs the kinetics of ACh release. This effect is reversed by muscarine. Methoctramine does not affect either the excitatory nerve terminal current or the presynaptic Ca²⁺ currents. These results support a novel hypothesis according to which depolarization initiates release by relieving a tonic block produced by the M2 receptor and rebinding of ACh to the M2 receptor terminates release.

Phrenic-diaphragm preparation of knockout mice without functional M2 receptors were compared with those of wild type mice. Experimental manipulations that affected [Ca²⁺]_i greatly changed the amount, but not the kinetics of release in wild-type mice. In contrast, in the mutant mice, treatments that affected [Ca²⁺]_i affected both the amount and also the kinetics of release. The behavior of release in M2-Ko mice follows predictions from the Ca²⁺ hypothesis, while the behavior in wild type mice follows predictions of the Ca²⁺-voltage hypothesis.

MEDIATOPHORE NO LONGER AN ARTEFACT

M. Israel¹, Y. Dunant²

*¹Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS,
Gif-sur-Yvette, France, ²Département de Pharmacologie, Geneva,
Switzerland*

Recently, Peters et al. found a proteolipid (V0-V0) channel between two membranes undergoing fusion, the SNARES serving as guides to put them face to face (see Almers' comment in Nature (2001)). The hemi-channel could open upon Ca-calmodulin action. In previous works, a protein mediophore was found to be made of this same proteolipid. Mediophore reconstitution and expression in transfected cells was characterized by its essential property, the translocation of acetylcholine (ACh) triggered by Ca. Since transfected cells released ACh in the absence of vesicular transporter and since release was quantal, mediophore was likely to be a part of the release machinery. Moreover it explained the release of cytosolic ACh in preference to vesicular ACh. In such a model, mediophores close to the Ca-channels are synchronized by the calcium micro-domains. The mechanism may be disrupted if vesicles that take up Ca are not properly anchored after clostridial toxin action. We do not exclude that, for some synapses, a V0-V0 fusion pore may open, but this process does not seem necessary for quantal release at rapid synapses. We were pleased that Nature considered, following Almers, that it would be poetic if we were partially correct. Was poetry missing in our first submission which was Nature-ally and Science-tifically rejected? or did Mediophore-V0 change too much synaptic and brain physiology?

NEUROTRANSMITTER PHENOTYPE SWITCH IN DEVELOPING NEURONS – GENOMIC AND PROTEOMIC APPROACHES

Michal Linial, Yaniv Bledi, Yoel Bogoch

*Department of Biological Sciences, Life Sciences Institute,
The Hebrew University, Jerusalem, Israel*

P19 cells are embryonal carcinoma cells that serve as a model for studying differentiation processes including commitment to cell lineage. We have studied P19 cells following activation of neuronal and glial differentiation. The potential of these cells to mature and efficiently release neurotransmitter (NT) was established in our lab. We discovered that several variables, most notably, cell density and various neurotrophic factors affects neuronal maturation, survival and most surprisingly, the choice of NT phenotype. We showed that P19 mature to functional cholinergic neurons but also to glutamatergic neurons according to culture manipulation protocols. Those changes are associated with changes in gene expression. A large-scale holistic view on gene expression was obtained by DNA chip technology. Several predicted but some novel signaling cascades are activated a result of manipulating the culture by neurotrophins and by elevating the cell density. We have observed that NT phenotype acquisition in P19 cells is mediated by cell-cell contact and hypothesized those cell surface proteins to be essential for consolidation of NT phenotype switch. We have used proteomics approach to focus on the relevant membranous proteins and applied comparative 1D and 2D analyses to isolate specific target proteins. Membranous proteins were isolated from P19 cells that were maintained in varying culturing conditions that support cholinergic vs. glutamatergic phenotypes. Membranes were collected at different time windows following neuronal induction. Differentially expressing proteins were excised and sent to Mass-spectrometry analysis. The analysis is based on MALDI and Electrospray mass spectrometry (ES-MS). Currently, over 30 proteins were successfully analyzed by such methodology. Using 2D gels we were able to improve detection level and could identify relatively low expressing proteins. Some proteins were identified multiple times in independent experiments. Most intriguing proteins are a variant of Drebrin – a putative dendritic-shaping molecule; Prohibitin that signifies post-mitotic cells; and several cytoskeletal-signaling molecules. Surprisingly, we observed marked changes in expression of a large group of heat shock proteins and their regulators along maturation of the neurons. The importance of a global unbiased proteomics view on developing neurons will be discussed.

GENETIC DISSECTION OF SYNAPTIC FUNCTION IN DROSOPHILA

T.L. Schwarz

*Division of Neuroscience, Children's Hospital and Harvard Medical
School, Boston, MA, USA*

Many questions of synaptic cell biology can be addressed by the study of mutations in model systems. This lab has used *Drosophila* genetics to elucidate the function of synaptic proteins. Mutations in neuronal-synaptobrevin, a vesicular SNARE protein, demonstrate distinct mechanistic requirements for minis and evoked release. By substituting related SNAREs, we have tested the specificity of trafficking and the hypothesis that SNAREs may uniquely target an individual vesicle class to the appropriate target membrane. Mutations of the C2A domain of synaptotagmin have also been examined and demonstrate that calcium-binding by this domain is not essential for calcium-dependent transmission. To facilitate the identification of new components of the axon and terminal, we have devised a novel method to screen for synaptic defects. By genetic manipulations, flies are screened that are heterozygous for mutations but whose eyes are completely homozygous for the mutation. Synaptic mutations are then recovered by screening for blind flies with characteristic electrophysiological defects. A gene called *milton* was isolated in this manner and is important for intracellular transport of organelles to synaptic terminals. Intracellular transport is thought to require adapter proteins for molecular motors in order to identify and bind specific cargoes, determine destinations, and anchor cargoes after transport. *Milton* appears to link kinesin to mitochondria. Mitochondria are completely absent from the *milton* photoreceptor nerve terminal and axon, but present and apparently functional in the cell body. *Milton* is present on mitochondria and is associated with kinesin heavy chain. *Milton* contains significant homology to the Huntingtin-binding domain of Huntingtin-associated protein 1 (HAP1). We propose that *Milton* is a mitochondrion-specific kinesin adapter protein required for axonal transport of mitochondria.

REGULATION OF CHOLINERGIC GENE EXPRESSION BY NRSF/REST

M. Shimojo, L.B. Hersh

University of Kentucky, Lexington, KY, USA

The cholinergic gene locus contains the genes for both the biosynthetic enzyme choline acetyltransferase and the vesicular acetylcholine transporter. Within the 5' region of the gene is found the 21 base-pair NRSE/RE-1 sequence to which the transcriptional repressor Neuron Restrictive Silencer Factor (NRSE)/RE-1 Silencing Factor (REST) binds and silences the gene. Although the cholinergic gene is active in PC12 cells, in a mutant PC12 cell line lacking protein kinase A the cholinergic gene is repressed. Although both wild type and mutant PC12 cells express NRSF/REST, only wild type cells express a neuron specific truncated form called REST4. We have found REST4 can form a hetero-oligomer with NRSF/REST and block its binding to the NRSE. We have found that REST4 can be trafficked to the nucleus and that this requires the participation of zinc finger domains. We have uncovered signals in REST4 (and presumably NRSF/REST) that are responsible for targeting to the nucleus, for entry into the nucleus, and for release into the nucleus. These will be described.

GENETIC REGULATION OF CHOLINERGIC NEUROTRANSMITTER PHENOTYPES

P.M. Salvaterra, M-H. Lee, S. Song

*Department of Neuroscience, Beckman Research Institute of the City of
Hope, Duarte, CA, USA*

The neurotransmitter phenotype of cholinergic neurons is determined primarily at the transcriptional level and involves controlling expression of the cholinergic gene locus. This locus is comprised of an unusual arrangement of both the choline acetyltransferase and vesicular acetylcholine transporter gene functions. We have established a model for cholinergic locus regulation in *Drosophila* primarily by analyzing transgenic animals with different parts of the 5' flanking DNA driving expression of functional or visible reporter genes. Our results imply that different subsets of cholinergic neurons regulate cholinergic locus expression separately and independently.

"Essential" expression, primarily in CNS neurons, seems to depend in part on the action of the POU domain transcription factor Nubbin. We have now obtained evidence that expression in many PNS olfactory neuron is dependent upon the action of another POU transcription factor, *Acj6* (a *Brn 3* homologue). In addition to the cholinergic locus, other genes are certain to be characteristic of the cholinergic phenotype. Some may be common to all types of cholinergic neurons such as the high affinity choline transporter, while others may define subsets of cholinergic neurons with characteristic functions. We have initiated a search for these other "cholinergic" genes using RNA isolated from sorted cholinergic neurons hybridized to gene microarrays.

*Supported by grants from the NIH-NINDS and the French Foundation for
Alzheimer's Research.*

COMPARATIVE STRUCTURAL STUDIES ON CONJUGATES OF TORPEDO CALIFORNICA AND HUMAN ACETYLCHOLINESTERASES WITH ORGANOPHOSPHATE NERVE AGENTS

J.L. Sussman¹, C.B. Millard², G. Koeltner¹, G. Kryger¹, M. Harel¹,
H. Greenblatt¹, H. Dvir^{1,2}, P. Bar-On², V. Neduva¹, K. Giles^{1,2}, A. Ordentlich¹,
Y. Segall¹, N. Ariel¹, D. Barak¹, B. Velan³, A. Shafferman³,
I. Toker², I. Silman²

*Depts of Structural Biology¹ and Neurobiology², Weizmann Institute of Science,
Rehovot³ Israel Institute for Biological Research, Ness Ziona, Israel*

Detailed understanding of structure-function relationships in the inhibition of AChE by OP nerve agents is a prerequisite for developing prophylactic and therapeutic approaches for treating nerve-agent intoxication. Knowledge of the 3D structures of native AChE and of relevant OP conjugates is essential for this. Our determination of the 3D structure of *Torpedo californica* AChE, followed by solution of the 3D structures of its 'aged' conjugates with sarin, soman and DFP, of both its 'aged' and 'non-aged' conjugates with VX, and of its conjugate with tabun has furthered this objective.

The 'aged' conjugates of TcAChE with sarin and soman display essentially identical 3D structures, thus providing structural models for the transition state during the deacylation step with acetylcholine as substrate.

Comparison of the native TcAChE structure and of the 'non-aged' and 'aged' conjugates with VX demonstrate reversible movement of the catalytic histidine. This movement involves a shift in hydrogen bonding from E327 to E199, which has been postulated to participate in substrate and OP reactions.

The 3D structures of the rhAChE/FAS-II complex with DFP, sarin and VX have been determined. Significant differences were observed relative to the corresponding TcAChE conjugates. It is premature to assign these differences to the bound FAS-II, which makes direct contact with the backbones of residues contributing to the acyl pocket, or to inherent species differences in plasticity of the acyl pocket. Such differences may also affect relative susceptibilities of electric organ and mammalian AChEs to other covalent inhibitors, such as PMSF and the anti-Alzheimer drug, rivastigmine.

CRYSTAL STRUCTURE OF RECOMBINANT HUMAN BUTYRYLCHOLINESTERASE: NEW INSIGHTS INTO THE CATALYTIC MECHANISMS OF CHOLINESTERASES

Y. Nicolet, F. Nachon, P. Masson, O. Lockridge, J.-C. Fontecilla-Camps

CRSSA, Département de Toxicologie, Unité d'Enzymologie,
La Tronche cedex, France, University of Nebraska Medical Center,
Eppley Institute, Omaha, NE, USA, Institut de Biologie Structurale, LCCP,
Grenoble cedex, France

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two related enzymes with different substrate and inhibitor specificities. BChE hydrolyzes a large variety of ester-containing drugs such as cocaine and scavenges organophosphorous and carbamate toxic esters. Mutants of BuChE capable of hydrolyzing organophosphates have been designed. However, their activity needs to be improved to be of operational interest for prophylaxis and/or treatment of nerve agent poisoning, and decontamination purposes.

Unlike AChE, no X-ray structure of BChE is known, mainly because of the high glycosylation content of natural BChE, preventing crystal growth. Therefore, most structure studies of BChE, have relied on homology models built from the *Torpedo californica* AChE structure. Although these models have been instrumental in understanding some aspects of the AChE and BChE specificity differences, they are not accurate enough as templates for the rational design of mutants with particular catalytic features.

We recently crystallized a recombinant monomeric low-glycosylated form of human butyrylcholinesterase. The structure was solved at 2.0 Å resolution by molecular replacement using the *Torpedo californica* AChE structure as a starting model. Both enzyme structures are very similar. However, the active site of BChE presents an unexpected feature which may change the current interpretation of the molecular mechanisms of cholinesterases.

SURPRISING FINDINGS FROM THE FUNCTIONAL ANALYSIS OF HUMAN ACETYLCHOLINESTERASE ADDUCTS OF ALZHEIMER'S DISEASE DRUGS

A. Ordentlich¹, C. Kronman¹, D. Barak², N. Ariel¹, D. Kaplan¹, B. Velan¹, A. Shafferman¹

¹Department of Biochemistry and Molecular Genetics, ²Department of Organic Chemistry, Israel Institute for Biological Research, Ness Ziona, Israel

Determination of the 3D-structure of acetylcholinesterase (AChE) of *Torpedo californica* over a decade ago and more recently that of human enzyme together with extensive targeted mutagenesis of the mammalian AChEs led to a fine mapping of the multiple functional subsites within the active center of the enzyme. Our library of single and multiple human AChE mutants defining the various subsites was used to kinetically analyze interactions with various AChE inhibitors including tacrine (Cognex), huperzine-A, rivastigmine (Excellon), physostigmine, pyridostigmine, E2020 (Aricept, Donepezil) and galanthamine which are considered or currently in use for the treatment of Alzheimer's disease (AD). Such functional analysis characterized the key domains within the active center that are essential for accommodation of these prototypic inhibitors. Furthermore it allowed defining major structural features of the individual inhibitors that determine affinity and specificity for the enzyme. Some important and unexpected interactions were revealed by the functional analysis that could have not been anticipated from the 3D-structure of inhibitor-AChE complexes. These findings emphasize the importance of complementing the structural data with functional characterization of biological target molecules. Thus it appears that screening of lead compounds with a library of human AChE mutants may be a very useful and cost effective way for structure-based design and development of new therapeutics for AD.

This work was supported by the U.S. Army Research and Development Command, Contract DAMD17-00-C-0021 (to A.S.).

INTRINSIC TRYPTOPHAN FLUORESCENCE OF CHOLINESTERASES: DIRECT, NON-PERTURBING MONITORING OF ENZYME-LIGAND INTERACTIONS

Z. Radic¹, E. Kim, P. Taylor

University of California at San Diego, Department of Pharmacology,
La Jolla, CA, USA

Intrinsic fluorescence of acetylcholinesterases (AChE, EC 3.1.1.7) and butyrylcholinesterases (BuChE, EC 3.1.1.8) was investigated to monitor enzyme-ligand interactions. The 10-13 tryptophans of cholinesterases emit fluorescence in 330 - 340 nm range upon excitation with UV light. Stern-Volmer analysis of collisional quenching of mouse AChE fluorescence by NaI, in the absence and presence of inhibitory peptide fasciculin 2, indicates that tryptophans of the AChE active center gorge contribute disproportionately to the overall fluorescence. Binding of non-fluorescent ligands, nonabsorbing in 330-340 nm range and incapable of resonance energy transfer, such as decamethonium, BW286c51, edrophonium, ethopropazine, acetylcholine and choline, quench AChE and BuChE fluorescence. However, binding of fasciculin 2 and carbamoylation of the active serine increase mouse AChE fluorescence intensity about 15%, thus suggesting conformational change involving triptophanes in the interaction. The rate of the conformational change appears faster than equilibration of reversible enzyme-inhibitor complexes thus allowing monitoring of inhibitor association and dissociation reactions in the millisecond time frame. Use of catalytically inactive mouse AChE S203A mutant allows for direct titration of AChE with acetylcholine revealing two binding sites for the substrate, one with K_d of ~80 μM and the other one of ~30 mM. In addition, measurements of rates of quenching of intrinsic AChE fluorescence upon association of edrophonium with mouse wild-type and H4471 mutant AChE demonstrated a pH dependence consistent with the protonation state of H447 of the catalytic triad. In conclusion, monitoring of intrinsic tryptophan fluorescence of AChEs and BuChEs, is a sensitive and non-perturbing method of studying interactions of these enzymes with variety of nonfluorescent ligands. (Supported by grants from the NIH and DAMDC).

UNFOLDING AND FOLDING OF *TORPEDO CALIFORNICA* ACETYLCHOLINESTERASE

L. Weiner, C.B. Millard, I. Shin, E. Roth, D. Kreimer, I. Silman

Weizmann Institute of Science, Rehovot, Israel

Chemical modification of Cys231 in *TcAChE* by various sulfhydryl reagents results in its conversion to one of two principal states. One of these states, produced by disulfides and by alkylating agents, is a stable state which displays typical features of a partially unfolded molten globule (MG) state, based on CD, intrinsic fluorescence and ANS binding. The second state, produced by mercury derivatives and the natural thiosulfinate, allicin, which has spectroscopic characteristics very close to those of the native (N) state is metastable: at room temperature it converts spontaneously with a half-life of ~1.5 h to the MG state. We named this state quasi-native (N*). Demodification of *TcAChE* in the N* state by glutathione or cysteine causes rapid release of the bound reagent, and concomitant recovery of most of the enzymatic activity. In contrast, similar demodification of the MG enzyme produces no detectable recovery of enzymic activity. Transition to the MG state of N *TcAChE*, as well as of enzyme in the N* state, is greatly accelerated in the presence of phosphatidylcholine liposomes. Introduction of osmolytes (glycerol, sucrose, tetramethyl N-oxide), as well as of certain divalent cations (Mg²⁺, Ca²⁺, Mn²⁺) retards transition of both the N and N* states to the MG state. The mechanisms underlying transition of the N and N* states to the MG state both in the absence and presence of liposomes, as well as the stabilisation induced by chemical chaperons, will be discussed according to the following scheme: N ⇌ N* ⇌ MG

SCAVENGER PROTECTION AGAINST ORGANOPHOSPHATES BY CHOLINESTERASES

B.P. Doctor¹, A. Saxena¹, M.T. Clark¹, Y. Rosenberg², D.M. Maxwell¹, D.E. Lenz³, Y. Ashani⁴

¹Walter Reed Army Institute of Research, Silver Spring, MD, USA,

²Procell, Rockville, MD, USA, ³Department of Biochemistry and Pharmacology, USAMRIID, Edgewood, MD, USA,

⁴IIBR, Ness-Ziona, Israel

The failure of current pharmacological approaches to provide complete protection against toxic organophosphorus (OP) compounds have lead to the development of enzyme bioscavengers to reduce their toxicity. Among the enzymes that hold promise as scavengers, significant advances have been made using cholinesterases (ChEs). These enzymes were found to be effective bioscavengers against a variety of OPs in rodents as well as in non-human primates. Pretreatment of rhesus monkeys with fetal bovine serum (FBS) AChE or horse/human serum butyrylcholinesterase (BChE) protected them against 5 LD50 of soman challenge. Monkeys pre-treated with ChEs, were devoid of any behavioral incapacitation after such soman challenges. Bioscavengers can afford protection against mortality, as well as all of the adverse physiological/behavioral effects of nerve agent exposure. The use of bioscavengers would provide a capability for extended protection against a wide spectrum of nerve agents and eliminate the need for extensive post-exposure therapy. We have recently isolated and purified several gram quantities of human serum BChE from Cohn fraction IV and have evaluated its use as a bioscavenger for safety and efficacy. We plan to examine human BChE for its lack of an autoimmune response. Preliminary results with purified monkey serum BChE, when re-administered into monkeys twice at one month interval, indicated that most of the enzyme remained in circulation after both injections. Also no antibody was detected against homologous BChE after the repeated administration of monkey enzyme. The mean retention time of this homologous BChE was approximately two weeks. These results bode well for the use of plasma-derived human BChE as a pretreatment drug for humans.

PRESYNAPTIC INHIBITION OF CENTRAL ACETYLCHOLINE RELEASE WITH A₁ LIGANDS: PREVENTION OF CHOLINERGIC CRISIS

H.P.M. van Helden¹, T.J.H. Bueters¹, B. Groen¹, M. Danhof², A.P. IJzerman²

¹Department of Pharmacology, TNO Prins Maurits Laboratory, Rijswijk,

²LAC/DR, Leiden, The Netherlands

The objective of this study is to explore a new strategy to counteract organophosphate poisoning via adenosine A₁ receptor-mediated inhibition of acetylcholine (ACh) release.

I.v. administration of the A₁ full agonist N⁶-cyclopentyladenosine (CPA) 1 min after a s.c. soman, sarin or tabun poisoning (1.5 – 2LD50), resulted in (i) prevention/ postponement of chewing, salivation, convulsive activity, and respiratory distress, (ii) improvement of 24 h survival, (iii) low levels of extracellular ACh in the brain. Since partial A₁ agonists have only limited cardiovascular effects compared to full agonists, the effectiveness of these partial ligands in attenuating the central evoked ACh release was examined in two ways:

- (1) Perfused striatal neuronal synaptosomes from which ³H-ACh release was evoked by 4-aminopyridine in the presence or absence of A₁ receptor agonists.
- (2) Inverse brain microdialysis with a dialysis probe in the corpus striatum.

In the striatal synaptosomes, CPA caused a dose-dependent inhibition ($I_{max} = 42 \pm 11\%$) of the evoked [³H]-ACh release. 3'-deoxy-CPA (3DCPA), 8-propylamino-CPA (8PCPA) and 8-butylamino-CPA (8BCPA) attenuated the release to a similar extent, although at 10-fold higher dosages. Microdialysis experiments demonstrated similar results on the inhibition of ACh release by CPA and its analogues, although higher I_{max} values were obtained. In conclusion, CPA, 3DCPA and the 8-alkyl analogues reduced the ACh release in the striatal area both *in vitro* and *in situ* in a concentration-dependent manner and appeared to be as efficacious as the full agonist CPA, suggesting that central inhibition of ACh release may be obtained without the above-mentioned side effects. The present results encourage further investigation of the therapeutic potential of these low efficacy agonists for the treatment of organophosphate poisoning.

A COMPLEX ARRAY OF POST-TRANSLATION MODIFICATIONS DETERMINES THE CIRCULATORY LONGEVITY OF ACETYLCHOLINESTERASE IN A HIERARCHICAL MANNER

C. Kronman, T. Chitlaru, A. Ordentlich, B. Velan, A. Shafferman

Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona, Israel

The post-translation modifications of the mature forms of acetylcholinesterase (AChE) include processing at the glycosylation and enzyme subunit assembly level. While these modifications do not appear to affect catalysis, they have a remarkable impact on the pharmacokinetic behavior of the enzyme which may limit its use as a therapeutic bioscavenger. Biochemical and genetic intervention in several post-translation processes of AChE were carried out. The various AChE glycoproteins generated were subjected to subunit assembly determination, extensive MALDI-TOF structural analysis of their N-Glycans, and pharmacokinetic profiling. Accordingly, asialylated forms of AChE displayed a very rapid removal rate (mean residence time, MRT= ~3 min) regardless of their subunit-assembly state, while partially sialylated forms displayed a longer residence time (MRT= 60-100 min) which could be further improved by their conversion into tetramers (MRT= ~200 min). Fully sialylated AChE exhibited an enhanced mean residence time (~200 min) as compared to the partially sialylated forms, which could be further extended by tetramerization (MRT= 740-1340 min). Unraveling this hierarchical linkage between post-translation modifications and circulatory retention of AChEs, allowed us to generate recombinant products indistinguishable from the native long-lived plasma-derived enzyme. These findings may be extended to other glycoproteins with pharmaceutical potential.

This work was supported by the U.S. Army Research and Development Command, Contract DAMD17-00-C-0021 (to A.S.).

MEASURING CEREBRAL ACETYLCHOLINE ESTERASE ACTIVITY IN ALZHEIMER DEMENTIA BY PET FUNCTIONAL PARAMETRIC IMAGING

K. Herholz, G. Zündorf, B. Bauer, S. Weisenbach, W.-D. Heiss

Max-Planck-Institute and Neurological Dept., University Hospital, Köln, Germany

Background and Purpose: In-vivo measurement of the expression of acetylcholine esterase (AChE) is of primary interest in dementia because of the crucial role of the cholinergic system in memory and attention. [¹¹C]-labeled N-methyl-4-piperidyl-acetate (¹¹C-MP4A) is a tracer that is highly specific for AChE and has kinetic properties that are favorable for measuring cortical AChE activity^{1,2}. Using this tracer, we describe the results of a new method for standardized parametric images in Alzheimer dementia (AD)^{3,4}.

Methods: We studied eleven patients with probable AD (NINCDS-ADRDA criteria, mean age 68.2 years, mean mini mental status score 21.2) and six normal control subjects. Subjects were examined with dynamic PET (Siemens/CTI EXACT) in 3D-mode over 60 minutes after injection of 550-740 MBq ¹¹C-MP4A i.v. A dynamic sequence of 6x30 seconds, 2x60 seconds, 2x150 seconds, 10x300 seconds scans was used after acquisition of a 10 min transmission scan with 3 Ge-68 rods. Images were normalized to stereotactic space. Parametric images of tracer hydrolysis by AChE (k_1) were generated by a recently developed voxel-wise dynamic curve fitting procedure using the putamen as a reference tissue. To localize the regions that demonstrate a significant change of AChE activity two sample t-tests were performed voxel by voxel using SPM99.

Results: Mean cortical k_1 values of late onset subjects ($k_1 = 0.0725 \pm 0.0090 \text{ min}^{-1}$) and early onset AD subjects ($k_1 = 0.0686 \pm 0.0072 \text{ min}^{-1}$) were significantly reduced compared to controls ($k_1 = 0.1019 \pm 0.0055 \text{ min}^{-1}$). The corresponding significance levels were $p = 4.6 \cdot 10^{-4}$ and $p = 1.2 \cdot 10^{-5}$, respectively. The most significant reduction of AChE activity was located in inferior temporal cortex.

Conclusion: Noninvasive measurement of local cerebral AChE activity with PET is possible and can be applied in a clinical setting. Severe reduction of AChE activity is present in mild to moderate AD of early and late onset. This technique has the potential to contribute to differential diagnosis of dementia, to measure cerebral AChE inhibition by drugs, and to identify individuals with particularly severe cholinergic deficits who may benefit most from treatment with cholinergic agents.

References

1. H. Namba et al., *Eur. J. Nucl. Med.* 26, 135-143 (1999).
2. K. Herholz et al., *J. Neural Transm.* 12, 1457-1468 (2000).
3. K. Herholz et al., *Eur. J. Nucl. Med.* 28, 472-477 (2001).
4. G. Zündorf et al., In *Brain imaging using PET*, M. Senda, Y. Kimura, P. Herscovitch, Eds. Academic Press, San Diego, Ca., in press.

BLOOD-BRAIN BARRIER DISRUPTION IS ASSOCIATED WITH ABNORMAL CORTICAL THETA RHYTHM GENERATION: THE POTENTIAL INVOLVEMENT OF ACETYLCHOLINESTERASE

E. Avivi¹, I. Shelef², H. Golan³, A. Korn¹, O. Tomkins¹, L. Pavlovsky¹,
A. Friedman¹

¹*Departments of Physiology and Neurosurgery, ²Radiology and ³Nuclear Medicine, Ben Gurion University and Soroka Medical Center, Zlotowski Center of Neuroscience, Beersheva, Israel*

Numerous pathological diseases of the central nervous system have been reported to involve perturbation of the integrity of the Blood-brain-barrier (BBB). Although the mechanisms underlying BBB disruption and the possible risk-factors are still unknown, previous studies point to possible AChE involvement. We have developed a fast technique for the analysis of computerized tomography (CT) images, thus enabling the screening of patients for perturbation of the BBB. Our data show frequent, focal or diffuse BBB disruption in a wide range of neurological disorders. Clinical data, blood and CSF biochemical analyses pointed to general stress-associated mechanisms as the common denominator, rather than a single disease process. Moreover, the radiological data suggest that BBB dysfunction may persist for at least several weeks. To explore the possible effects of BBB disruption on cortical activity, patients with focal BBB disruption were examined with high density 128 channel electroencephalography. Abnormally high power theta activity was noted in all patients with BBB disruption. In 9 out of 13 patients examined, low-resolution electrotomography (LORETA), localized the abnormal slow wave origin in the same brain region as the anatomical BBB lesion. In a separate animal model, electrophysiological recordings in mouse brain slices suggested cholinergic-mediated enhanced glutamatergic activity as an important contributor to abnormal cortical activity. Our data suggests that persistent BBB disruption may underlie abnormal brain activity and resultant neurological disorders.

CONGENITAL MYASTHENIC SYNDROMES (CMS): MULTIPLE MOLECULAR TARGETS AT THE NEUROMUSCULAR JUNCTION

A.G. Engel, K. Ohno, S.M. Sine

Mayo Clinic, Rochester, MN, USA

Congenital myasthenic syndromes (CMS) stem from defects in presynaptic, synaptic, and postsynaptic proteins. Deeper understanding of disease mechanisms and precise classification of the CMS requires counts of AChR per endplate (EP), light and electron microscopy analysis of EP morphology, electrophysiology assessment of EP function in vitro, mutation analysis if the preceding point to a candidate gene or protein, and expression studies to confirm the pathogenicity of identified mutations. Presynaptic CMS stem from defects that curtail the evoked release of acetylcholine (ACh) quanta or ACh resynthesis. Insufficient resynthesis of ACh is now known to be caused by mutations that reduce the expression, catalytic efficiency, or both, of choline acetyltransferase. The synaptic CMS are caused by mutations in the collagenic tail subunit (ColQ) of the EP species of acetylcholinesterase that prevent ColQ from associating with catalytic subunits or from insertion into the synaptic basal lamina. The postsynaptic CMS arise from kinetic or low-expressor mutations in AChR subunits, or mutations in rapsyn or plectin. Kinetic AChR mutations affect affinity, gating efficiency, or both, and result in slow- and fast-channel syndromes that respectively prolong or curtail channel opening events. Low expressor AChR mutations are concentrated in the epsilon subunit owing to partial compensation by the fetal gamma subunit. Null mutations in both alleles of other AChR subunits are likely lethal due to absence of a substituting subunit. Rapsyn mutations identified to date reduce AChR expression by impairing recruitment of AChR to rapsyn.

CHEMICAL KINETICS PARAMETERS AND RECEPTOR DEGRADATION RATES AT THE NEUROMUSCULAR JUNCTION

Edwin E. Salpeter

Cornell University, New York, USA

Two projects will be described which were started by Mika Salpeter before her tragic and untimely death. One project presents an exhaustive catalog of modeling results for miniature endplate currents (mepc) for many choices of numerical values for chemical kinetics (and other) input parameters. The catalog will be available to the e-mail reader. In relation to Myasthenia Gravis, one can follow changes in mepc risetime and amplitude as cleft width and AChR site density are varied separately or together.

The second project concerns acetylcholine receptor (AChR) site densities (SD) at the neuromuscular junction during two transitional periods, denervation and reinnervation. EM autoradiography was used. The simplest theoretical model predicts an upward transient in SD during denervation and a downward transient during reinnervation. The former is observed, but not the latter.

Degradation experiments suggest that a form of AChR with a 3.5 day half-life is inserted during a five day reinnervation period (faster than intact adult AChR; slower than embryonic AChR). It is inserted at a large enough rate to keep SD constant, so that mepc amplitude can already go to the full adult value during this transition period.

Credited to NIH Grant NS09315.

ANTIBODIES TO ACETYLCHOLINE RECEPTORS AND MUSK IN MYASTHENIA GRAVIS AND RELATED DISORDERS

A. Vincent

Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

Antibodies to acetylcholine receptors (AChR) cause the autoimmune disease, myasthenia gravis (MG), which results in muscle weakness and fatigue. About 15% of patients with classical MG do not have antibodies to the muscle AChR. In vitro their serum antibodies appear to act by disturbing an intracellular signalling pathway that leads to AChR phosphorylation. In collaboration with Dr Werner Hoch (Max Planck Institute for Developmental Biology) we showed that 70% of MG patients without AChR antibodies, have antibodies to the muscle specific kinase, MuSK (Hoch et al Nat Med 7, 365, 2001). These antibodies can inhibit agrin-induced, MuSK-dependent, AChR clustering in vitro. Thus these antibodies are potentially pathogenic and are likely to reduce the number of AChRs or alter the structural integrity of the neuromuscular junction. A small proportion of MG women transfer a transient MG to their babies, so called neonatal MG. Very occasionally, the baby is born with severe multiple joint contractures. Studies with Dr David Beeson, using adult and fetal AChR expressed in *Xenopus* oocytes, demonstrated the presence in these mothers of antibodies that specifically inhibit the fetal isoform of the AChR. Moreover, if the human antibodies to fetal AChR are injected into pregnant mice, the mouse fetuses become paralysed and develop joint contractures. Antibodies to voltage-gated calcium channels and voltage-gated potassium channels are involved in other neurological disorders. These antibodies can also affect the autonomic and central nervous system causing a range of different symptoms including autonomic dysfunction, cerebellar ataxia, or sleep and memory disorders. Autoantibodies to specific ion channels at cholinergic synapses are responsible for a range of different neurological disorders.

INHIBITORY EFFECTS OF MUSCARINIC RECEPTOR AUTOANTIBODIES ON PARASYMPATHETIC NEUROTRANSMISSION IN SJÖGREN'S SYNDROME

S.A. Waterman, S. Lester, T.P. Gordon, M. Rischmueller

Departments of Rheumatology, Flinders Medical Centre, The Queen Elizabeth Hospital, Adelaide, SA, Australia

Sjögren's syndrome (SS) is an autoimmune disorder characterized by dry eyes and mouth (sicca syndrome) and lymphocytic infiltration of lacrimal and salivary glands. Abnormalities of parasympathetic neurotransmission may contribute to the glandular dysfunction. We developed a functional assay to investigate autoantibody-mediated effects on parasympathetic neurotransmission.

Serum and purified IgG was obtained from patients with primary and secondary SS and controls. Contraction of isolated murine bladder strips in response to stimulation of M₃-muscarinic receptors by carbachol or by endogenous acetylcholine released from postganglionic parasympathetic nerves was measured before and after the addition of patient serum or IgG. Sera from 5/9 patients with primary SS and 6/6 patients with secondary SS inhibited carbachol-evoked bladder contraction by approximately 50%, as well as the action of neuronally released acetylcholine at M₃-muscarinic receptors. Sera from 7/8 healthy individuals and 8/8 disease controls had no effect. Anti-muscarinic receptor activity was localised in the IgG fraction. The autoantibodies were associated with bladder symptoms and other autonomic features.

Conclusion: Autoantibodies that act as antagonists at M₃-muscarinic receptors on smooth muscle occur in patients with primary and secondary SS. These autoantibodies appear to contribute to sicca symptoms and may explain associated features of autonomic dysfunction. Development of screening assays will enable study of their broader clinical relevance.

IMMUNOTHERAPY OF MYASTHENIA GRAVIS: ANTIGEN-SPECIFIC MUCOSAL TOLERANCE AND ANTAGONISTS OF KEY CYTOKINES AND COSTIMULATORY FACTORS

Miriam C. Souroujon^{1,2}, Sin-Hyeog Im¹, Sara Fuchs¹

¹Department of Immunology, The Weizmann Institute of Science, Rehovot,

²The Open University, Tel-Aviv, Israel

Developing immunotherapeutic approaches for myasthenia gravis has been our goal for many years. Mucosal administration of recombinant fragments corresponding to the human acetylcholine receptor (AChR) α -subunit suppresses ongoing experimental autoimmune myasthenia gravis (EAMG) in rats. Treated animals exhibit a Th1 to Th2/Th3 shift in their cytokine profile and down-regulation of costimulatory factors. In severely affected rats, this antigen-specific approach may need to be supported by direct modulation of key cytokines and costimulatory factors known to be involved in the pathogenesis of EAMG. To address this question, myasthenic rats were injected by antibodies either to the proinflammatory cytokine IL-18 or to the costimulatory factor CD40L. These treatments act via different mechanisms but both lead to alleviation of clinical symptoms even when given at the chronic phase of EAMG. Both impaired AChR-specific Th1 cell differentiation with no effect on Th2-type responses, and treatment by anti-IL-18 antibodies led to elevation of the Th3-type suppressive cytokine, TGF- β and to generation of regulatory cells. Anti-CD40L administration led to a significant decrease in humoral responses, whereas anti-IL-18 treatment affected mainly cellular responses to AChR. The most significant suppressive effect of both treatments was observed 2-3 weeks after initiation of treatment and was later diminished, implying that blockade of either IL-18 or CD40L may not be sufficient to suppress chronic myasthenia. We therefore suggest that antagonists to key cytokines and/or costimulatory factors be used in conjunction with antigen-specific treatments such as mucosal administration of AChR recombinant fragments.

THE ALPHA7 NACHR L250T MUTATION IN MICE: A MODEL FOR EPILEPSY IN MEN

A. Orr-Urtreger¹, R.A. Sack¹, M. Kedmi¹, A. Harmelin³, Z. Gil²

¹Genetic Institute, ²Department of Otolaryngology and Head Neck Surgery, Tel Aviv Sourasky Medical Center, Tel Aviv, ³Weizmann Institute of Science, Rehovot, Israel

Alpha7 nicotinic acetylcholine receptors (nAChRs) are sparsely distributed throughout the peripheral and central nervous systems. Several studies have suggested that central alpha7 nicotinic receptors may influence sensitivity to nicotine-induced seizures in mice. In order to investigate the role alpha7 in seizure, we tested heterozygous mice with a threonine for leucine substitution at position 250 (+/L250T) within the channel domain of the alpha7 subunit. This mutation was previously shown to cause a partial 'gain of function' in this receptor subtype (i.e., increased current amplitude and agonist affinity and decreased desensitization of the receptor). We demonstrated that administration of low doses of nicotine to alpha7 +/L250T mice significantly increased the sensitivity to nicotine-induced seizures. EEG recordings and spectral analysis of these mutants showed high amplitude rhythmic activity that was in correlation with the behavioral changes induced by nicotine. Pretreatment with the alpha7 nicotinic receptor antagonist methyllycaconitine (MLA) inhibited the seizures induced by nicotine. Our data suggest that the alpha7 subunit is involved in seizure generation. Since the alpha7 L250T mutation resembles the human alpha4 and beta2 nAChR mutations found in autosomal dominant nocturnal frontal lobe epilepsy, the +/L250T animals may serve as an important tool to study the mechanism and treatment of human epilepsy.

THE MOLECULAR NEUROBIOLOGY OF ACETYLCHOLINESTERASE VARIANTS: FROM STRESSFUL INSULTS TO ANTISENSE INTERVENTION

H. Soreq, D. Glick

Institute of Life Sciences, The Hebrew University of Jerusalem, Israel

The early discovery of the acetylcholine hydrolyzing enzyme, acetylcholinesterase (AChE) and its function in terminating cholinergic neurotransmission made it the focus of intense research for much of the past century. More recent studies on the complexity of AChE gene regulation and the accumulating evidence for some of the long-suspected "non-classical" actions of this protein call for exploring the molecular neurobiology of AChE splicing variants. To this end, we have combined transgenic animal models with genomic and cell culture studies aimed at revealing the enzymatic and morphogenic roles of 3'-alternative splicing products of the human AChE gene under normal and disease conditions. There are three, not one AChE variants, each with a different C-terminal peptide, capacity for multimeric assembly and potentially distinct non-classical function(s). Both stressful stimuli and exposure to AChE inhibitors induce transcriptional activation and shift alternative splicing toward overproduction of the normally rare "readthrough" variant, AChE-R. This may be beneficial for suppressing the initial insult, but detrimental under long-term conditions. Partially 2'-oxymethylated oligodeoxynucleotides capable of ameliorating AChE-R overproduction emerge as promising research tools for proving the putative involvement of AChE-R in such detrimental consequences, as well as potential therapeutic agents. Specific advantages of such agents stem from their degree of variant selectivity, which enables maintenance of cholinergic neurotransmission while preventing the stress-induced imbalance among AChE variants.

ACETYLCHOLINESTERASE FACILITATES AMYLOID DEPOSITION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

T. Rees¹, P. Hammond¹, S. Younkin¹, H. Soreq², S. Brimijoin¹

¹Department of Molecular Pharmacology, Mayo Clinic, Rochester, MN, USA, ²Hebrew University, Jerusalem, Israel

Alzheimer-type amyloid plaques contain acetylcholinesterase (AChE), among other proteins. In vitro, AChE associates with beta-amyloid (A-beta) and hastens formation of insoluble amyloid fibrils in a manner that might promote plaque formation (Alvarez et al., 1998). To determine if this effect is clinically relevant for Alzheimer's disease, we investigated Tg2576 mice, which overexpress human amyloid precursor protein (hAPP) and develop plaques at about 9 months (Hsiao et al., 1996). Tg2576 mice were crossed with mice harboring a human AChE transgene (Beerli et al., 1996) to yield F1 hybrids that overexpress both hAChE and hAPP in brain. By 6 months, the cerebral cortex of the hybrid mice demonstrated plaques reacting with thioflavin S and with antibodies to A-beta 1-40 and 1-42. Plaque burden at this stage was modest (~125 plaques per hemisphere) and quantitative ELISA showed very little SDS-insoluble, formic acid extractable A-beta, but levels of SDS-soluble A-beta were about 25% above control. By 9 months of age, plaques had become larger and more numerous, while formic acid extractable A-beta 1-40 and 1-42 had reached levels double those in age-matched controls. These results suggest that direct interactions with AChE can promote amyloid deposition in brain, initially as an atypically loose, detergent soluble matrix. Therefore, drugs designed to target protein-protein interactions between AChE and A-beta should be tested for potential to retard progression of Alzheimer's disease.

NEURODEGENERATIVE PROCESSES IN ALZHEIMER'S DISEASE

N.C. Inestrosa, G.V. De Ferrari, J.L. Garrido, A. Alvarez, M. Bronfman

Centro de Regulacion Celular y Patologia, Depto. Biologia Celular y Molecular, P. Universidad Catolica de Chile, Santiago, Chile

Alzheimer's disease (AD) is a progressive dementia paralleled by selective neuronal death, which is probably caused by the cytotoxic effect of the amyloid beta-peptide (Abeta). We have previously shown that the senile-plaque component acetylcholinesterase (AChE) induces amyloid fibril formation, forms macromolecular complexes (Abeta-AChE) being incorporated into the growing fibrils, and that such complexes increased amyloid neurotoxicity. Therefore, we have searched for molecular changes induced by Abeta-AChE complexes, both in neuronal cells in vitro and in rats injected in the dorsal hippocampus with Abeta/Abeta-AChE complexes, as an in vivo model of the disease. Here, we will discuss that Abeta/Abeta-AChE neurotoxicity results in the destabilization of endogenous levels of key components of the Wnt signal transduction pathway. Moreover, activation of this signaling cascade either with lithium, protein kinase C (PKC) agonists or with conditioned media containing the Wnt-3a ligand, induce survival of post-mitotic neurons against neurotoxicity and rescue the deficit in spatial learning induced by such cytotoxic agents. Given that it has been recently observed that

nicotinic receptors (viz. alpha-7nR), which may signal through PKC isoenzymes, binds Abeta with nanomolar affinities, we have also examined the ability of its agonists/antagonists (i.e. alpha-bungarotoxin) to modulate Abeta/Abeta-AChE neurotoxicity. Our results suggest that Abeta/Abeta-AChE dependent neurotoxicity results in loss of function of Wnt signaling components and indicate that compounds that mimic this signaling cascade may be candidates for therapeutic intervention in Alzheimer's patients.

This work was supported by grants FONDAP N 1389001 and MIFAB N 2398969 NCI is recipient of a Presidential Chair in Science from the Chilean Government and a John Simon Guggenheim Foundation Fellow.

TRANSCRIPTIONAL CONTROL OF THE CHOLINERGIC GENE LOCUS (CGL): A MOSAIC MODEL FOR REGULATION OF THE CHOLINERGIC PHENOTYPE

L.E. Eiden¹, B. Schuetz², M. Goerdes³, C. Depboylu³, M.K.H. Schafer³, E. Weihe³

¹Section on Molecular Neuroscience, NIMH, Bethesda MD, USA, ²Laboratory on Molecular Neurobiology, Clinic for Psychiatry, Bonn University Clinical Center, Bonn, ³Institute of Anatomy and Cell Biology, Marburg, Germany

While VACHT and ChAT transcription are coordinated, low levels of R-exon expression indicate independent transcription of each gene from the rodent CGL *in vivo* (Hahn et al., J. Mol. Neurosci. 9; 223, 1997; Schuetz et al., Neuroscience 104; 633, 2001). Co-expression of VACHT and ChAT with the high-affinity choline transporter, transcribed from a separate gene (Okuda et al., Nat. Neurosci. 3; 120, 2000), is much less tightly coupled in certain cholinergic neurons. An 8.7 kb fragment of the human CGL extending ~2 kb past the VACHT gene is sufficient for hVACHT expression in somatomotor neurons of transgenic mice (Schuetz et al., Neuroscience 96; 707, 2000). An additional 2.5 kb downstream fragment is required for diencephalic expression (Schuetz et al., this meeting), in contrast to expression from the murine CGL in transgenic mice (Naciff & Dedman, J. Neurochem. 72; 17, 1999).

Co-transmitter expression in cholinergic neurons is also highly species-specific. Cholinergic neurons of the rat stellate ganglion co-express VACHT and TH before birth, and at sweat gland terminals soon after. TH expression is switched off in the adult rat, but persists in the adult mouse and primate. Human sweat gland terminals are also positive for VMAT2, consistent with human 'noradrenergic sweating'. Cyclooxygenase (COX), rate-limiting for prostaglandin biosynthesis, is co-expressed in cholinergic nucleus basalis neurons in primates, and irreversibly down-modulated in simian AIDS. Thus, prostaglandins may be involved in inflammation-associated cholinergic dysfunction.

These findings suggest a 'mosaic' model for CGL transcription that allows fine-tuning of cholinergic traits, and differential co-transmitter expression, in different types of cholinergic neurons.

NEURONAL NICOTINIC RECEPTORS, ALLOSTERIC POTENTIATING LIGANDS (apls), AND ENDOGENOUS METABOLITES: IMPLICATIONS FOR TREATMENT OF ALZHEIMER'S DISEASE (ad).

E.X. Albuquerque^{1,2}, M.D. Santos², M. Alkondon¹, E.K. Moon¹, A. Maelicke³

¹Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD, USA, ²UFRD, Rio de Janeiro, Brazil, ³Johannes-Gutenberg University Medical School, Mainz, Germany

The severity of symptoms in AD, a progressive neurodegenerative disorder that afflicts millions worldwide, is closely related to the extent of reduction of nicotinic cholinergic activity in the brain. Thus, augmenting nicotinic functions has emerged as a promising therapeutic approach for treatment of AD. Research from our laboratories has demonstrated that nicotinic receptor (nAChR) activity can be increased by substances referred to as APLs. Galantamine, a weak anticholinesterase, is the prototypic APL. By increasing activity of presynaptic nAChRs, galantamine causes sustained facilitation of synaptic transmission in the brain. Methamidophos, Metrifonate, Tacrine and Donepezil, in contrast, are anticholinesterase agents devoid of APL activity, and they facilitate synaptic transmission only transiently. Recent studies also revealed that (i) kynurenic acid, a tryptophan metabolite whose levels are increased in the brain of AD patients, is a non-competitive antagonist at alpha7 nAChRs (IC50, 7 micromolar) and increases alpha4beta2 nAChR expression in the brain, and (ii) acetyl-L-carnitine, a metabolite whose levels are decreased in the brain of AD patients, potentiates nAChR activation by nicotinic agonists. These findings lay the groundwork for development of new therapeutic avenues for treatment of patients with AD.

Support: Janssen Pharmaceutical Research Foundation, U.S.A.M.R.D.C DAMD-17-95-C-5063, USPHS grant NS25296.

THE RATIONALE FOR USING GALANTAMINE TO TREAT DIFFERENT DEMENTIA TYPES

A. Maelicke¹, S. Lilienfeld², C. Grantham³

¹Laboratory of Molecular Neurobiology, Institute of Physiology, University of Mainz, Germany, ²The R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ, USA, ³Division of Janssen Pharmaceutica N.V., Beerse, Belgium

Objective: Considerable evidence has established that impaired cholinergic neurotransmission is central to Alzheimer's disease (AD). Cholinergic deficits related to nicotinic acetylcholine receptors (nAChR) coupled with degeneration of neuronal circuits are now believed to contribute to cognitive impairment across a range of dementias, including vascular dementia (VaD). Here, we present a rationale for using drugs that affect nAChR activity to treat dementia. **Methods:** We examined preclinical and clinical evidence to define the nature and contribution of cholinergic impairment to symptoms of AD and other dementias to determine a rational treatment strategy. **Results:** Human and animal models indicate that, regardless of the underlying cause of dementia, cognitive deficits involve decreased nicotinic cholinergic neurotransmission and reduced numbers of nAChR. Cholinergic function is compromised in animal models of VaD. Studies have shown reduced binding of nAChR ligands in the cortex and hippocampus of spontaneously hypertensive stroke-prone rats. Decreased choline acetyltransferase activity has also been observed in the cortex, hippocampus and striatum of VaD patients compared with controls. Patients with Binswanger or multiple small infarct VaD subtypes have lower cerebrospinal fluid concentrations of acetylcholine. Galantamine (Reminyl(R)), an acetylcholinesterase (AChE) inhibitor that allosterically modulates nAChR, may be particularly useful for treating various dementias. Recent research has shown that, as well as having broad, long-term efficacy in AD, galantamine is effective in patients with VaD and AD with concomitant cerebrovascular disease. **Conclusion:** Drugs like galantamine, with a nicotinic mode of action in addition to AChE inhibitory activity, provide an effective treatment option for various types of dementia.

GENETIC DISSECTION OF AN ACETYLCHOLINE RECEPTOR INVOLVED IN NEURONAL DEGENERATION

M. Treinin, S. Halevi, L. Yassin

Department of Physiology, Hebrew University – Hadassah Medical School, Jerusalem, Israel

The *C. elegans* DEG-3 gene codes for a subunit of a nicotinic acetylcholine receptor that can mutate to cause neuronal degeneration. The degeneration causing mutation, *deg-3(u662)* is a gain of function mutation, affecting a residue in transmembrane domain II, that apparently interferes with receptor desensitization. In order to identify genes needed for DEG-3 activity we screened for mutations that suppress the behavioral defects associated with the *deg-3(u662)* mutation. This screen led to the identification of mutations in three genes: *deg-3* itself, *des-2* an acetylcholine receptor subunit, and *ric-3* a novel gene specifically required for the maturation of acetylcholine receptors. Co-expression of these genes in *Xenopus* oocytes shows that DES-2 is essential for DEG-3 channel activity and that RIC-3 affects channel activity both quantitatively and qualitatively. The reconstruction of DEG-3 channel activity in oocytes also suggests that *deg-3(u662)*-induced degenerations are a result of constitutive activity of the mutant channel in the presence of physiological choline concentrations; choline is an agonist of the DEG-3 channel. High calcium permeability of this channel may also contribute to the degeneration process. Thus genetic analysis has identified elements affecting acetylcholine receptor activity and is providing insights into the degeneration process.

MOLECULAR AND FUNCTIONAL DIVERSITY IN NICOTINIC ACETYLCHOLINE RECEPTOR GENE FAMILIES OF *C. ELEGANS* AND *D. MELANOGASTER*

David B Sattelle

MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, UK

Genetics, genomics and electrophysiology are transforming our understanding of the largest known gene family of nicotinic acetylcholine receptor (nAChR) subunits in *C. elegans* (27 members) and the exclusively neuronal nAChR gene family in *D. melanogaster* (10 members). In *C. elegans*, several genetic screens have enabled identification of nAChR subunits, along with novel proteins that act upstream and downstream of functional nAChRs. The *C. elegans* genome project has identified many new candidate nAChR subunits and the calculated electrostatic potential energy profiles for the M2 channel-lining regions predict considerable functional diversity. The respective roles of subunits are under investigation using forward and reverse genetics. Electrophysiological and reporter gene studies have demonstrated roles for particular subunits in levamisole-sensitive muscle nAChRs and a role for nAChRs in pharyngeal pumping. Recombinant homomeric and heteromeric *C. elegans* nAChRs have been expressed in *Xenopus laevis* oocytes. In *D. melanogaster*, three new nAChR subunits have been cloned, one of which shows multiple variant transcripts arising from alternative splicing and A-to-I pre-mRNA editing. Thus, studies on the genetic model organisms *C. elegans* and *D. melanogaster* have revealed different routes to generating molecular and functional diversity in the nAChR gene family and are providing new insights into the *in vivo* functions of individual family members.

A COMMON AGONIST AND POTENTIATOR FOR $\alpha 7$ NICOTINIC AND 5-HT₃ SEROTONIN RECEPTORS

R. Zwart, L. Broad, C. Felthouse, K. Pearson, G. McPhie, E. Sher

Eli Lilly and Company Ltd., Windlesham, UK

$\alpha 7$ nicotinic receptors ($\alpha 7$ nAChRs) as well as serotonin 5-HT₃ receptors (5-HT₃Rs) belong to the family of ligand-gated ion channels. These two receptors exhibit some cross-pharmacology, e.g. high concentrations of the $\alpha 7$ nAChR agonist nicotine inhibit 5-HT₃R-mediated responses, and high concentrations of the 5-HT₃R agonist 5-HT inhibit $\alpha 7$ nAChR-mediated responses.

Using voltage clamp and calcium imaging techniques, we are studying the pharmacological properties of 5-HT₃Rs in murine N1E-115 neuroblastoma cells and of cloned human 5-HT₃Rs and $\alpha 7$ nAChRs expressed in *Xenopus* oocytes. We have found that a recently described $\alpha 7$ nAChR agonist (Philips et al., Astra Arcus USA, patent WO99/03859) is activating human $\alpha 7$ nAChRs expressed in oocytes. However, the same compound also activates human 5-HT₃Rs. The EC₅₀, *nH* and E_{max} are 2.2 μ M, 0.8 and 83% for $\alpha 7$ nAChRs, and 1.4 μ M, 1.8 and 66% for 5-HT₃Rs, respectively. In the presence of 30 μ M dH β E, this agonist still induces robust inward currents and raises [Ca²⁺]_i in N1E-115 cells. These responses are blocked by the selective 5-HT₃R antagonist MDL72222.

5-Hydroxyindole (5-HI) potentiated 5-HT₃R- as well as $\alpha 7$ nAChR-mediated responses. The threshold concentration of 5-HI to potentiate both types of receptor is 100 μ M, and maximum potentiation is observed at 10 mM 5-HI. On both types of receptor, 1 mM 5-HI shifts the agonist concentration-effect curve towards lower agonist concentrations and enhances the maximum effect of both ACh and 5-HT.

In this study, the general notion that 5-HT₃Rs and $\alpha 7$ nAChRs share some pharmacological properties is extended by the finding that both types of receptor share a common agonist and a common potentiator.

BETA-AMYLOIDS, TAU HYPERPHOSPHORYLATION AND COGNITION ARE BENEFICIALLY AFFECTED BY M1 MUSCARINIC AGONISTS - PERSPECTIVES IN ALZHEIMER'S DISEASE TREATMENT

A. Fisher¹, Z. Pittel¹, R. Brandeis¹, R. Haring¹, N. Bar-Ner¹, H. Sonego¹, I. Marcovitch¹, N. Natan¹, N. Maestre-Frances², N. Bons²

¹Israel Institute for Biological Research, Ness Ziona, Israel.

²Université Montpellier II, Montpellier, France

M1 agonists from the *AF* series [AF102B (prescribed in USA for Sjogren's Syndrome), AF150(S) & AF267B] - *i*) restored cognitive impairments in several animal models for Alzheimer's disease (AD) with an excellent safety margin; *ii*) elevated the non-amyloidogenic amyloid precursor protein (alpha-APPs) levels; *iii*) attenuated vicious cycles induced by *beta*-amyloid (*Abeta*), and inhibited *Abeta*- and oxidative stress-induced apoptosis; and *iv*) decreased *tau* protein hyperphosphorylation *in vitro* and *in vivo* [review: Fisher Jpn J Pharmacol 84: 101, 2000]. Unlike M1 agonists, nicotinic agonists and cholinesterase inhibitors increased *tau* hyperphosphorylation [Hellstrom-Lindhal et al J Neurochem 74, 777, 2000]. In aged microcebes (a natural model for AD; Bons, Alz. Res 1, 83, 1995), prolonged treatment with AF150(S) restored cognitive and behavioral impairments and decreased *tau* hyperphosphorylation, paired helical filaments and astrogliosis [Bons, Maestre-Frances and Fisher, unpublished]. In rabbits, with *Abeta* sequence identical to the human *Abeta*, AF267B & AF150(S) decreased CSF *Abeta*_(1-42 & 1-40), while AF102B reduced *Abeta*₍₁₋₄₀₎ [Beach et al Brain Res. 905, 220, 2001]. Finally AF102B decreased CSF *Abeta*_(total) in AD patients [Nitsch et al Ann Neurol. 48, 913, 2000]. In summary, M1 agonists may represent a unique therapy in AD due to their combined beneficial effects on the three major hallmarks of AD - cholinergic hypofunction, *Abeta* and hyperphosphorylated *tau*.

Support in part by the Institute for the Study of Aging, New York, USA.

CROSS -TALK BETWEEN APOLIPOPROTEIN E THE AMYLOID PRECURSOR PROTEIN AND BRAIN INFLAMMATION

D.M. Michaelson¹, S. Meilin¹, G. Ophir¹, Y. Ezra¹, L. Oron¹, S.M. Beni², E. Shohami²

¹Department of Neurobiochemistry, Tel-Aviv University, ²Jerusalem, Israel

Transgenic mice expressing either human apoE3 or apoE4 on a null mouse apoE background were employed to investigate the role of isoform-specific interactions between apoE, the amyloid precursor protein (APP) and brain inflammatory processes in the phenotypic expression of the pathophysiological effects of the apoE genotype

Immunoblot measurements of the brain levels of APP and of its soluble fragment APPs, revealed that they were lowest in the apoE4 transgenic mice. Closed head injury increased the levels of brain APPs of all mice groups. This effect was markedly and significantly larger in the apoE3 transgenic mice than in the other mice groups.

LPS was injected to the mice i.c.v. and the resulting levels of activation of microglia and astrocytes in the brains of the different mice groups were monitored immunohistochemically. This revealed that LPS treatment of 12 months old mice triggered astrogliosis in the apoE-deficient and the apoE4 transgenic mice, but not in the control and apoE3 transgenic mice. In contrast, LPS dependent microglial activation was not significantly affected by either apoE deficiency or the human apoE transgenes.

In conclusion, the present findings show that APP metabolism and brain inflammatory processes are modulated *in vivo* in an isoform specific manner by apoE, and suggest that the pathological effects of apoE4 in Alzheimer's disease may be mediated by similar cross talk interactions.

CHOLINERGIC DEFICITS AND NON-COGNITIVE BEHAVIOURAL CHANGES IN PATIENTS WITH DEMENTIA

P.T. Francis, C.P.L.-H. Chen, M.M. Esiri, J. Keene

Centre for Neuroscience, King's College London, UK, Department of Neurology, Singapore Hospital, Singapore, Department of Neuropathology, Oxford University, UK, Department of Psychiatry, Oxford University, UK

Non-cognitive behavioural changes such as depression, aggressive behaviour, psychosis and overactivity occur frequently in patients with dementia, in addition to cognitive impairment, and often determine the need for institutionalisation. The biochemical basis of such changes is poorly understood. Clinical trial data indicate that cholinomimetics improve non-cognitive behaviours. We wished to investigate the relationship between markers of the cholinergic and neurotransmitter system and non-cognitive behavioural symptoms assessed during the course of dementing illness.

Brains from 46 patients with dementia (36 with Alzheimer's disease, AD, and 10 with mixed or other dementias) were examined together with 32 normal controls.

The patients with dementia had been evaluated every 4 months, often over several years, for cognitive performance (Mini-Mental State Examination) and behaviour (Present Behavioral Examination). Choline acetyltransferase activity (ChAT) and density (Bmax) of muscarinic M1 and M2 receptors in frontal and temporal cortex were studied by radioligand binding protocols. None of the patients were receiving cholinomimetic drugs. ChAT activity and M2 density, but not other neurochemical markers, were reduced in AD compared with controls. Loss of ChAT activity correlated with cognitive impairment. Lowered ChAT activity also correlated with increasing overactivity in patients with dementia in both frontal and temporal cortex while M2 receptor density was increased in frontal cortex of AD patients with delusions and in temporal cortex of those with hallucination compared to patients without psychotic symptoms. Disturbance of the cholinergic system may underlie both cognitive and some non-cognitive behavioural changes in dementia, providing a basis for rational therapy.

"Supported by The Wellcome Trust"

ACETYLCHOLINESTERASE REGULATION IN SKELETAL MUSCLES

J. Sketelj, N. Crne-Finderle, P. Pregelj

Institute of Pathophysiology, School of Medicine, University of Ljubljana, Slovenia

Muscle acetylcholinesterase (AChE) is concentrated in the rat neuromuscular junctions (NMJ) as the asymmetric (A) molecular form, composed of the catalytic tetramers and the collagen Q (CollQ) subunit. In immature muscles, CollQ is expressed all along the fibers. It is segregated to the NMJs in mature fast muscles but not in the slow soleus muscle. Distribution of the A AChE forms behaves accordingly to the CollQ expression. Regulation of the expression of the extrajunctional A AChE forms in muscle fibers is a nerve dependent phenomenon. A forms of AChE in the NMJ are bound to the basal lamina largely by ionic interactions involving Ca²⁺, but about 1/3 is bound more firmly. Expression of the catalytic subunit of AChE in muscle fibers is higher in the fast than in the slow rat muscles, in accordance with about 3-4-fold difference in AChE mRNA level. This is probably due to different patterns of muscle activation of the two types of muscles because the level of AChE mRNA is high in phasically activated fibers and decreases after low-frequency tonic stimulation. The soleus is an antigravity muscle and differs from fast muscles also in regard to loadbearing, but muscle load is not decisive for differences in AChE expression. However, thyroid hormones have an enhancing effect on the level of muscle AChE mRNA. Hyperthyroidism increased AChE mRNA levels in the slow muscle (but not in the fast), whereas hypothyroidism decreased AChE expression in fast and slow muscles.

CHOLINERGIC CORTICAL TERMINATIONS ESTABLISH CLASSICAL SYNAPSES AND UNDERGO AGE-RELATED ATROPHY

A. Claudio Cuello, Paolo Turrini, Maria A. Casu, Tak Pan Wong,
Yves De Koninck, Alfredo Ribeiro da Silva

*Departments of Pharmacology and Therapeutics, Anatomy and Cell
Biology, and Medicine, McGill University, Montreal, Quebec, Canada*

For many years it has been assumed that cortical cholinergic synapses communicate with target-neurons in a "non-synaptic" fashion (volume transmission). We have recently re-examined this problem at the ultrastructural level by applying a highly reliable marker of presynaptic cholinergic boutons: antibodies against the vesicular acetyl choline transporter (VACHT). Our investigations demonstrate that - contrary to the above view - cholinergic neurons in the cerebral cortex establish classical synapses. Thus, the electron microscopical analysis of lamina V in the rat cerebral cortex revealed that the majority of cholinergic boutons (i.e. those immunoreactive to VACHT) established typical axo-dendritic synapses of the symmetric type, and are rarely seen in cell soma or dendritic spines. When they are seen in the latter cases, they were asymmetric synaptic contacts. Combining intracellular labelling of tissue slices with high-resolution immunocytochemistry, we observed a preferential relationship of cholinergic boutons with dendritic shafts of pyramidal neurons. We further investigated the fate of cholinergic synapses during aging. This revealed that the age-related loss of cholinergic boutons precedes that of the overall pre-synaptic population. In addition, cholinergic pre-synaptic boutons undergo a marked atrophy with aging. These manifestations of cortical cholinergic synaptic disconnection should be the major reason for cholinergic participation in age-dependent cognitive decline.

Supported by C'IHR grant # MT-14494 to A.C.C.

ACTIVATION OF THE CHOLINERGIC SYSTEM DURING COGNITIVE PROCESSES

G. Pepeu, M.G. Giovannini, J. Cangioli, M.B. Passani

Department of Pharmacology, University of Florence, Italy

The nucleus basalis-cortical-amygdalar, and septo-hippocampal cholinergic pathways play a critical role in information processing. However, the cognitive events in which they are activated are not yet fully identified. Therefore, the aim of our investigations was to study the relationship between ACh release from cerebral cortex, hippocampus and basolateral amygdala and behaviours in adult male rats, used to be handled, during exploration of a novel environment (arena), habituation, and fear conditioned response. ACh release was investigated by microdialysis coupled to HPLC detection and quantification.

Three 5 min exposures to an arena, with 60 min intervals, were accompanied by a rapid two fold increase in cortical ACh release followed by a prompt drop to basal levels when the rats returned to the home cage. If the exposures lasted 30 min, cortical and hippocampal ACh outputs were significantly smaller during the second exposure than during the first. Motor activity increased mainly during the first exploration of the arena and was significantly correlated to cortical but not hippocampal ACh release. These results indicate that the cortical and hippocampal cholinergic pathways are activated during exploration of a novel environment, probably subserving arousal and attention, but not when habituation takes place. In the amygdala, the facilitation of fear response consolidation by H3 histamine receptor agonists and the impairment by H3 receptor antagonist are associated with an increase or decrease, respectively, in ACh release, suggesting its role in fear memory formation.

Supported by MURST (PRIN 1998 and 2000).

DEVELOPMENT OF CHOLINERGIC PROJECTIONS TO CORTEX: POSSIBLE ROLE OF NEUROTROPHINS IN TARGET SELECTION

R. Robertson, J. Yu

University of California, Irvine, CA, USA

The goal of this project is to understand how growing cholinergic axons identify and form synaptic contacts with their target cells; the septal cholinergic projection pattern to the dentate gyrus was used as a model system. In the first set of studies, distributions of cells expressing mRNA for selected neurotrophins were studied in developing rats and mice using either autoradiographic or immunocytochemical techniques. These studies demonstrated that NT-3 and BDNF expression develops in dentate gyrus granule cells in spatial and temporal patterns that parallel, and slightly precede, the patterns of ingrowth of septally derived cholinergic axons. Patterns of NGF mRNA expression were not similar to patterns of cholinergic ingrowth. In a second set of studies, organotypic slice cultures were prepared using slices of medial septum paired with a slice of hippocampus, including the dentate gyrus. Normal co-cultures made from tissue from rats or wild-type mice display organotypic patterns of septal cholinergic ingrowth into the dentate gyrus. In contrast, chimeric co-cultures made from septum from wild type mice and hippocampus from NT-3 null mice show profuse cholinergic axonal growth into the hippocampus, but the organotypic pattern of axons in the dentate gyrus is not formed. Instead, AChE positive axons in these cultures grow directly through the dentate gyrus without forming terminal branches in the molecular layer. These data suggest that expression of NT-3 by dentate gyrus granule cells serves to attract or retain septum-derived cholinergic projections to the inner molecular layer of the dentate gyrus.

PRECLINICAL AND CLINICAL STUDIES ON THE ROLE OF MUSCARINIC RECEPTORS IN THE PHARMACOTHERAPY OF SCHIZOPHRENIA

F.P. Bymaster¹, A. Shekhar², K.W. Perry¹, K. Rasmussen¹, D. McKinzie¹,
C.C. Felder¹

¹Neuroscience Research Division, Lilly Research Laboratories,
Indianapolis, IN, USA. ²Department of Psychiatry, Indiana University,
Indianapolis, IN, USA

Emerging preclinical and clinical evidence supports a role for cholinergic muscarinic receptors (MR) for novel pharmacotherapy of schizophrenia. Anatomical studies indicate that (MR) are uniquely distributed to modulate sensory information, dopaminergic and glutamatergic neurotransmission which may be dysregulated in psychosis. Hyperactivity of dopamine in limbic tracts and hypoactivity in cortical regions is hypothesized to cause positive and negative symptoms of schizophrenia, respectively, and MR have been shown to modulate these dopamine tracts. For example, the muscarinic M1/M4-preferring agonist xanomeline increases cortical extracellular dopamine and Fos expression, similar to effects of atypical antipsychotics. In electrophysiological studies, xanomeline with acute and chronic administration decreased firing of the mesocorticolimbic dopamine A10 tract, but not the motoric A9 tract. Behavioral investigations have shown that muscarinic agonists, like dopamine antagonists, inhibit dopamine-agonist-induced behaviors including hyperactivity, climbing and disruption of prepulse inhibition, models for positive symptoms. Knockout mice with ablated M4 receptors are hyperactive and hyperresponsive to dopamine D1 agonists, suggesting a dynamic balance between dopamine and M4 receptors. Mice with M1 receptors deleted have deficits in cognition and muscarinic agonist-induced in vivo phosphoinositide hydrolysis. Consistent with preclinical studies, preliminary clinical investigation indicates that xanomeline may be effective for pharmacotherapy of psychosis and promoting cognition in schizophrenia. Studies in knockout mice have shown that activation of the muscarinic M2 and M3 receptors produce the majority of parasympathomimetic side effects. Thus, we hypothesize that a combined M1 agonist to promote cognition and a M4 agonist for antipsychotic-like effects would treat the symptom domains of schizophrenia without causing parasympathomimetic side effects.

CENTRAL CHOLINERGIC NEURONS IN CULTURE: REGULATION OF SURVIVAL AND FUNCTION

M. Segal, N. Landman, V. Greenberger

*Department of Neurobiology, The Weizmann Institute of Science,
Rehovot, Israel*

Selective degeneration of basal forebrain cholinergic neurons has been associated with cognitive impairment seen in Alzheimer's disease. We examined the properties of cultured septal cholinergic neurons of the rat brain, grown in presence of growth factors or hippocampal neurons, which are innervated by cholinergic neurons *in vivo*. Individual cholinergic neurons, visualized by selective staining with a fluorescent antibody to the p-75 NGF-receptor and recorded with a patch pipette expressed spontaneous activity consisting of both excitatory and inhibitory synaptic currents. The presence of target hippocampal neurons, but not of NGF, enhanced spontaneous network activity in septal cultures. Exposure to NGF caused an increase in the size of cholinergic cell somata and primary dendrites, an effect that was only partially shared by the presence of hippocampal neurons. Exposure of cultures to glutamate agonists as well as to H₂O₂, resulted in a higher proportion of cell death among cholinergic vs. non-cholinergic neurons, indicating that the former neurons are more sensitive to neurotoxic insults. The role of afferent hippocampal neurons in neuroprotection is currently analyzed. These studies are expected to contribute to the understanding of the unique role of cholinergic neurons in cognitive aspects of the brain, and the unique properties of the septo-hippocampal cholinergic system.

Supported by a grant from the Alzheimers Association

HOW IS THE BRAIN SUPPLIED WITH CHOLINE, BUT PROTECTED AGAINST EXCESS CHOLINE?

K. Löffelholz, J. Klein

Department of Pharmacology, University of Mainz, Germany

In the brain, choline (Ch) is released from the cytoplasm of neuronal cells by depolarization, is taken up from the blood at elevated plasma levels (enhanced pharmacologically or nutritionally), and finally is catalytically released from ACh and from phospholipids. An excessively high extracellular level of Ch leads to nicotinic receptor activation or desensitization. However, the overall level of (Ch)_o is remarkably constant at about 4-6 μ M due to homeostatic mechanisms. Surplus Ch is removed from the brain by a net-release into the venous blood. K⁺-induced depolarization of synaptosomes caused the release of Ch due to the fact that the transmembrane Ch gradient follows the Nernst equation. However, in brain slices or in hippocampal tissue of adult rats (using the microdialysis technique) K⁺ failed to enhance (Ch)_o apparently due to mechanisms counteracting the K⁺-induced Ch release. In contrast, high K⁺ did elevate (Ch)_o in brain slices of one week old rats, which have not yet fully developed cellular uptake mechanisms. Brief electrical stimulation of cholinergic nerves caused a biphasic change of the synaptic Ch level: a transient increase (due to ACh hydrolysis) was followed by a long-lasting hemicholinium-sensitive decline. Long-lasting activation of cholinergic pathways by pharmacological or behavioral means lead to a deficit of synaptic Ch which became rate-limiting for ACh biosynthesis; the reduced ACh release was reversed by Ch supplementation. On the basis of the above analysis of Ch homeostasis, the significance of temporal and spatial fluctuations of the synaptic Ch level is still a matter of speculation.

SIGNALING PATHWAYS THAT REGULATE THE CHOLINERGIC GENE LOCUS EXPRESSION

B. Berse¹, I. Lopez-Coviella², T. Mellott¹, B.E. Slack¹, M.T. Follettie³,
R.S. Thies³, L. Li¹, J.K. Blusztajn^{1,2}

¹Department of Pathology, ²Department of Psychiatry, Boston
University School of Medicine, Boston, MA, ³Genetics Institute, Inc.,
Cambridge, MA, USA

The overall goal of our studies is to reveal how cholinergic neurons acquire and maintain their neurotransmitter characteristics. The two genes that determine the cholinergic phenotype are choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter (VACHT). They occupy one genomic locus and are likely controlled by shared transcriptional mechanisms. We employed model cholinergic cell lines and embryonal spinal cord and septal neurons to investigate the regulation of ChAT and VACHT expression by ciliary neurotrophic factor (CNTF) and bone morphogenetic proteins (BMPs). By using pharmacological inhibitors of MAPK kinase (MEK1), as well as expression of recombinant constitutively activated MEK1, we demonstrated the negative effect of the MEK1 pathway on CNTF-induced cholinergic promoter activity. We reported previously that BMP-9 is a potent inducer of the cholinergic phenotype *in vitro* and *in vivo*. Transient transfection of reporter constructs into primary neurons from E14 mice revealed that BMP-9 activates transcription from the proximal - but not from the distal - promoter region of the cholinergic locus. In search of candidate intermediate transcription factors involved in this BMP action, we used DNA microarray technology (Affymetrix GeneChip) to analyze the changes in gene expression patterns caused by BMP-9. In rat and mouse E14 neuronal cultures, BMP-9 activated the expression of multiple genes, including growth factors, growth factor receptors, and transcription regulators. It is likely that the products of some of these BMP-9-induced genes are involved in the modulation of cholinergic gene locus expression. Supported by grants IIRG-00-2073 (ILC), AG09525 (JKB), and NS30791 (BES).

CONTROL OF ACETYLCHOLINE RELEASE UNDER STIMULATORY CONDITIONS BY ITS BIOSYNTHETIC PRECURSORS; GLUCOSE AND CHOLINE

J. Klein, S. Kopf, K. Loeffelholz

Department of Pharmacology, University of Mainz, Germany

We tested the effects of glucose and choline, the biosynthetic precursors of acetylcholine, on passive avoidance behaviour and hippocampal acetylcholine release measured by microdialysis in awake mice. Glucose (10 and 30 mg/kg) or choline chloride (6-60 mg/kg), given by intraperitoneal injection immediately after training, dose-dependently enhanced retention in an inhibitory avoidance task. Combinations of low doses of glucose (10 mg/kg) and choline chloride (20 mg/kg) which alone were submaximally effective significantly increased retention latencies in a synergistic manner, an effect which was sensitive to atropine (0.5 mg/kg). This beneficial effect vanished when higher doses of glucose or choline were combined. Basal hippocampal acetylcholine release in mice habituated to their environment was not affected by administration of glucose and choline. However, when hippocampal acetylcholine release was stimulated either by infusion of scopolamine (0.3 μ M) or by transferring the mice into a novel environment, the combination of glucose plus choline further increased acetylcholine release to a significant extent. We conclude that low doses of glucose and choline act synergistically to improve memory storage, an effect which is due to facilitation of acetylcholine release. This finding reinforces the view that central cholinergic functions are under certain conditions influenced by dietary intake of precursors.

TREATMENT OF DEMENTIA WITH CHOLINESTERASE INHIBITORS

Amos D. Korczyn

*Sieratzki Chair of Neurology, Tel-Aviv University Medical School,
Ramat Aviv, Israel*

Dementia is a heterogeneous group of disorders with several identifiable etiologies and mechanisms. It is becoming recognized that the border between Alzheimer's disease (AD) and the second most common cause of dementia, vascular dementia (VaD) is blurred, and a rational approach should direct itself to cardiovascular factors, such as hypertension, which could prevent AD as well as VD. The current treatment of patients with dementia includes primarily cholinesterase inhibitors. These agents (donepezil, rivastigmine, and galantamine) have a limited symptomatic effect, expressed on concentration, cognition and behavior, and affecting activities of daily living and independence. However, pathological and biochemical data also point to central cholinergic hypofunction in both disorders. Therefore, it is logical to examine the effect of ChEI's in VaD as well as in cases with mixed dementia.

Limited attempts to affect other parameters (non-cholinergic neurotransmitters and post-receptorial processes) have been limited and have largely failed.

ChEI's are of limited value for several reasons. They cause significant side effects, such as gastro-intestinal and cardio-vascular. Their potential value is also limited because they enhance the activity of the ever-declining acetylcholine. The use of muscarinic agonists in dementia has been limited. Reasons for failure of the previous studies will be outlined, and ways to overcome them will be suggested. In particular, the use of combined ChEI's with M₁ agonists will be supported.

GENDER DIFFERENCES IN THE ACTIONS OF CHOLINESTERASE INHIBITORS

M. Weinstock, R-H. Wang

*Department of Pharmacology, Hadassah Medical School, Hebrew
University, Jerusalem, Israel*

Introduction: Cholinesterase (ChE) inhibitors are currently the most effective treatment for Alzheimer's disease (AD). In normal elderly women and in those with AD, physostigmine produced a larger increase in plasma ACTH, and cortisol than in men, but blood levels did not differ. This study determined whether there was a sex difference in the effects of tacrine and rivastigmine and its relation to hormone levels. **Method:** The effects of tacrine and rivastigmine were compared on spatial memory impaired by scopolamine, body temperature and ChE activity in males and females.

Results: Both drugs caused significantly greater hypothermia, antagonism of scopolamine-induced memory impairment and ChE inhibition in the brain, but not in the heart or skeletal muscle in females than in males. The sex difference resulted from higher amounts of drug reaching the female brain. Orchidectomy increased drug activity and brain levels in males to that in females, while ovariectomy had no effect. Adrenalectomy increased brain levels of tacrine and activity in both sexes. Testosterone decreased brain levels and actions of ChE inhibitors both in castrated males and females.

Conclusions: The pharmacological effects of ChE inhibitors in the brain are greater in females than in males because testosterone reduces the amount of drugs reaching its target sites. It remains to be seen if women with AD are also more sensitive than men to the cognitive effects of these drugs.

CHOLINESTERASE INHIBITORS STABILIZE COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

Ezio Giacobini

*Department of Geriatrics, University of Geneva, School of Medicine,
Thonex-Geneva Switzerland*

Cholinesterase inhibitors (ChEI) are the only drugs approved in US and Europe for the indication of Alzheimer Disease (AD) treatment.

Long-lasting clinical efficacy of one year or longer produced by ChEI treatment in AD patient suggests that long-term effects of these drugs may not be solely symptomatic and related to an elevation of synaptic acetylcholine levels. Six most extensively clinically tested ChEI (tacrine, eptastigmine, donepezil, rivastigmine, metrifonate and galantamine) produce a stabilization of cognitive and non cognitive function. Data collected from over 8.000 cases demonstrate that patients responders to treatment with ChEI change little cognitively and behaviorally from the baseline at the beginning of the study for six-twelve months as compared to placebo treated patients. Long-term effects of one year or longer are seen using four different ChEI (donepezil, metrifonate, rivastigmine and galantamine). Additional clinical observations suggest non-symptomatic effects. Effects on APP metabolism and release in vitro and in vivo and on beta-amyloid toxicity and aggregation may contribute to long term clinical efficacy seen in AD patients treated with ChEI (1).

*1. Cholinesterase and Cholinesterase Inhibitors (2000) Ed. By E. Giacobini,
Martin Dunitz (London)*

AMYLOID PRECURSOR PROTEIN PROCESSING PROPERTIES OF THE NOVEL NEUROPROTECTIVE CHOLINESTERASE - MONOAMINE OXIDASE INHIBITOR, TV3326 AND ITS OPTICAL ITS-ISOMER, TV3279

M.B.H. Youdim¹, Merav Phalach-Yogev¹, Orit Bar-Am¹, Marta Weinstock²,
Tamar Amit¹

*¹Eve Topf and NPF Centers, Technion, Haifa and ²Department of Pharmacology,
Hebrew University, Jerusalem, Israel*

TV3326, (N-propargyl-(3R)-aminoindan-5-yl-ethyl, methyl carbamate) was developed in order to combine the neuroprotective effects of the antiParkinson drug, rasagiline, a selective inhibitor of monoamine oxidase (MAO)-B with the cholinesterase (ChE) inhibitory activity of rivastigmine as a potential treatment for Alzheimer's disease (AD). TV3326 is a cholinesterase and brain selective MAO A and B inhibitor, while its optical S-isomer, TV3279, has only cholinesterase inhibitory activity. Both drugs retain many of the cell culture and In Vivo neuroprotective-antiapoptotic properties of rasagiline and antagonize scopolamine-induced impairment in spatial memory. Reports indicate that cholinesterase inhibitors process amyloid precursor protein (APP) via α -secretase cleavage pathway. However their mechanism of action has not been fully established. Both TV3326 and TV3279 stimulated the release of the non-amyloidogenic α -secretase dependent soluble APP (sAPP) in PC12 and human neuroblastoma (SH-SY5Y) cells in a dose dependent (0.1-100 μ M) manner. This effect was blocked by hydroxamic acid-based metalloprotease inhibitor Ro-31-9790, indicating the mediation via α secretase cleavage. Using signal transduction inhibitors, we have shown that protein kinase C (PKC), mitogen-activated protein (MAP) kinase and tyrosine kinase dependent pathway may be involved in the enhancement release of sAPP by TV3326 and TV3279. In addition both drugs induced the phosphorylation of p44 and p42 MAP kinase and their effects were abolished by specific inhibitors of MAP kinase activation, PD98059 and U0126. Both drugs on oral chronic treatment (150 μ mole/kg/day X 14days) decrease rat and mice hippocampal APP holoprotein. Since the generation of sAPP precludes the formation of amyloidogenic derivatives, the demonstration that TV3326 and TV3279 can stimulate the non-amyloidogenic α -secretase pathway and decrease rat hippocampal full length APP suggests that these neuroprotective drugs may prevent the basic APP pathogenic mechanism underlying AD.

NOVEL BIFUNCTIONAL COMPOUNDS ELICITING CHOLINERGIC AND ANTI-INFLAMMATORY ACTIVITY FOR THE TREATMENT OF CNS IMPAIRMENTS

G. Amitai, R. Adani, I. Rabinovitz, G. Sod-Moriah, H. Meshulam

Division of Medicinal Chemistry, Israel Institute for Biological Research,
Ness Ziona, Israel

The development of new drugs for treatment of various CNS degenerative diseases such as dementia of Alzheimer's type (AD) is based mainly on the use of cholinergic compounds such as cholinesterase inhibitors (ChEI). It was shown that anti-inflammatory drugs, free radical scavengers and antioxidants could also serve for the amelioration of the inflammatory processes occurring in various CNS diseases. The clinically used drugs, which presently demonstrate efficacy in mild to moderate AD are ChEIs (e.g., Aricept, Exelon and Reminyl). Certain quaternary ChEIs (such as pyridostigmine (Pyr)) and polar non-steroidal anti-inflammatory NSAIDs (e.g. ibuprofen and diclofenac) could hardly cross the blood-brain barrier (BBB).

We have designed and synthesized a series of novel bifunctional compounds that contain covalently coupled cholinergic up-regulators (CURE) such as quaternary ChEIs, muscarinic and nicotinic agonists with various NSAIDs. These two moieties are coupled by a hydrophobic linker that renders permeability through the BBB.

This report is focused mainly on bifunctional NSAID-CURE compounds that are conjugates of NSAIDs with ChEIs. Most conjugates contain Pyr as ChEI moiety coupled via a hydrocarbon octyl linker (PO) to the following NSAIDs: ibuprofen (IBU), diclofenac (DICLO), indomethacin (INDO), aspirin (ASP) and naproxen (NAP). IBU-PO, DICLO-PO, INDO-PO, ASP-PO and NAP-PO inhibit both AChE and BChE with dissociation constants (K_i) and bimolecular kinetic rate constants (k_i) that range between 10^{-6} - 10^{-7} M and 10^5 - 10^6 M⁻¹ min⁻¹, respectively. Some of these bifunctional compounds also inhibit cyclooxygenase (COX I and COX II) at micromolar level. Thus, the NSAID-ChEI conjugates exert both anti-ChE and anti-COX activity at equi-molar concentrations even in their intact non-hydrolyzed form. The acute toxicity of these compounds is 10-20 fold lower than that of Pyr with LD₅₀ values that range at 50-100 mg/kg ip in mice. Anti-inflammatory activity of IBU-PO was examined in carrageenan-induced peripheral and CNS inflammation in rats. Pretreatment with IBU-PO (5mg/kg, ip) decreased significantly the rat paw edema level and whole brain edema. IBU-PO (5mg/kg, ip) increased by 8-fold the survival time of mice that were exposed to hypobaric hypoxia as compared to control animals. IBU-PO (5mg/kg, ip) decreased significantly the brain edema and improved the neurological severity score in closed head injury model in mice. These findings suggest that the new NSAID-ChEI bifunctional chimeras could be useful for treatment of CNS impairments such as cerebro-vascular dementia and for reducing the neuronal damage caused by either acute cerebral ischemia or closed head injury.

STRUCTURE AND ACTIVATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS

E.C. Hulme, Z.-L. Lu, M.S. Bee, C.A.M. Curtis

National Institute for Medical Research, London, UK

Systematic scanning mutagenesis has been applied to the transmembrane domain of the M1 muscarinic acetylcholine receptor. In addition to the negatively-charged aspartic acid residue in transmembrane (TM) helix 3, the aromatic rings of tyrosine residues in TM 3, 6 and 7 play a key part in binding acetylcholine (ACh), and triggering the conformational change which activates the receptor, while hydrogen bonding residues in TM 3, 5, 6 and 7 help to anchor ACh in the ground state binding site. Residues in TM 4 may contribute to a peripheral ligand docking site. Amino acids which make inter-helical contacts which help to stabilise the ground state of the receptor have been identified. These include the Asn and Tyr residues in the highly-conserved Asn-Pro-X-X-Tyr sequence in TM 7. The contacts made by these residues are probably rearranged during receptor activation, to produce a G-protein binding site. The results are presented in the context of a 3-dimensional model of the M1 muscarinic receptor, based on the structure of rhodopsin.

ROLES OF EXTERNAL LOOPS OF MUSCARINIC RECEPTORS IN INTERACTIONS BETWEEN N-METHYLSCOPOLAMINE AND ALLOSTERIC MODULATORS

Alena Krejci, Stanislav Tucek

Institute of Physiology, Academy of Sciences, Prague, Czech Republic

Allosteric modulators are known to modify the affinity of muscarinic receptors for orthosteric ligands but the sites of their attachment to receptors and the nature of the conformational change which they induce in receptors have been little clarified. It is assumed that they bind to a domain which is located more extracellularly than the orthosteric binding site. Neuromuscular blockers alcuronium and gallamine have a high affinity for and strong allosteric effects on the M2 receptors. Their affinity for and allosteric efficacy on the M3 subtype is much weaker.

We performed mutations or exchange of extracellular loops in the M3 receptors, trying to make this subtype more similar to the M2 subtype and to discover sites important for allosteric interactions. Transferring the second outer loop from M2 to M3 receptors enhanced the affinity for both alcuronium and gallamine, without changing their allosteric efficacy. On the other hand, M3 receptors in which the third outer loop had been modified so as to correspond to the same loop of the M2 receptors displayed not only higher affinities for both allosteric modulators, but also much stronger negative cooperativity between N-methylscopolamine (NMS) and gallamine. Alcuronium lost its negative effect on the binding of NMS (typical of M3 receptors) and acquired the ability to enhance the binding of NMS (positive cooperative action, typical of M2 receptors).

The third outer loop apparently plays important roles both in the high-affinity binding of gallamine and alcuronium and in the mechanism of their negative and positive allosteric effects.

GENERATION AND ANALYSIS OF MUSCARINIC ACETYLCHOLINE RECEPTOR KNOCKOUT MICE

A. Duttaroy¹, M. Yamada^{1,2}, J. Gomez¹, W. Zhang¹, R. Makita²,
T. Miyakawa³, F.P. Bymaster⁴, C.C. Felder⁵, C. Deng⁵, J. Wess¹

¹Laboratory of Bioorganic Chemistry, NIH-NIDDK, Bethesda, MD, USA.

²Laboratory for C/D, RIKEN Brain Science Institute, Wako-shi, Saitama, Japan.

³Center for Learning and Memory, MIT, Cambridge, MA, USA.

⁴Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA.

⁵Laboratory of Biochemistry and Metabolism, NIH-NIDDK, Bethesda, MD, USA

Identification of the physiological and pathophysiological roles of the individual muscarinic acetylcholine receptor subtypes (mAChRs; M1-M5) has proven a challenging task, primarily due to the lack of ligands endowed with a high degree of receptor subtype selectivity and the fact that most tissues and organs express multiple mAChRs. To circumvent these difficulties, we generated mutant mouse lines ('KO' mice) in which specific mAChR genes (M1, M2, M3, or M4) had been inactivated by gene targeting techniques. The different mAChR mutant mice and their wild-type littermates were subjected to a battery of physiological, pharmacological, behavioral, biochemical, and neurochemical tests. The M2 and M4 receptor mutant mice showed several striking phenotypes, as reported previously (Gomez et al., PNAS, 96, 1692 and 10483, 1999). M3 receptor KO mice displayed a significant decrease in food intake, associated with reduced body weight and low serum leptin and insulin levels, probably due to disruption of a hypothalamic cholinergic pathway involved in the regulation of appetite. Pharmacological analysis of M2 receptor single and M2/M4 receptor double KO mice indicated that muscarinic agonist-induced analgesic responses are mediated by both M2 and M4 receptors. Neurochemical studies showed that autoinhibition of ACh release is mediated primarily by M2 receptors in hippocampus and cerebral cortex, but predominantly by M4 receptors in the striatum. These results provide a rational basis for the development of novel muscarinic drugs.

STRUCTURAL AND FUNCTIONAL CONSERVATION OF SNARE COMPLEXES

D. Fasshauer, W. Antonin, M. Margittai, S. Pabst, R. Jahn

Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

The SNARE proteins syntaxin1, synaptobrevin2, and SNAP-25 play a key role during synaptic exocytosis. It was suggested that their assembly into stable membrane-bridging complexes gradually brings the membranes into close apposition. Thus, complex formation may provide the energy for initiating bilayer merger. The SNARE assembly pathway is only partly understood. Hence, we believe that detailed structural, kinetic, and thermodynamic investigations will bring a closer understanding of the function of SNARE proteins during membrane fusion. SNARE complex assembly can be described as a folding reaction, since it is accompanied by extensive structural rearrangements from less structured monomers to a tightly packed parallel four-helix bundle. Furthermore, a switch from a closed to an open conformation of syntaxin is thought to be a prerequisite for complex formation. We found that assembly and dissociation of the synaptic SNARE complex exhibits a marked hysteresis. It implies that assembly and dissociation do not follow the same path -probably due to a high energetic barrier between both native states.

Consequently, the ATPase NSF is necessary for disassembly. In addition, it suggests that SNARE assembly between two membranes is unidirectional - probably towards fusion. In an analogous study, we showed that an endosomal SNARE complex, composed of four SNARE proteins only distantly related to the synaptic SNAREs, has an almost identical structure to the synaptic complex. Moreover, the endosomal SNARE complex displayed a similar hysteresis. Taken together, our data suggest that SNARE function is conserved for all membrane fusion steps.

PRECLINICAL STUDIES OF GALANTAMINE USING A FORM OF ASSOCIATIVE LEARNING SEVERELY IMPAIRED IN ALZHEIMER'S DISEASE

Diana S. Woodruff-Pak

Temple University and Albert Einstein Healthcare Network, Philadelphia, PA, USA

Classical eyeblink conditioning is a well-characterized model paradigm for the study of the neurobiology of learning, memory, and aging which also has application in the differential diagnosis of neurodegenerative diseases. Studies of eyeblink conditioning in neurological patients along with brain imaging studies of conditioning in normal adults document parallels in the neural substrates of this form of associative learning in humans and non-human mammals. Disruption of septohippocampal cholinergic neurotransmission impairs acquisition of conditioned eyeblink responses and slows the rate of learning. Alzheimer's disease (AD) profoundly disrupts the hippocampal cholinergic system, and patients with AD consistently perform poorly in eyeblink conditioning. Some nicotinic acetylcholine receptor (nAChR) subtypes are lost in AD making the use of allosteric modulation of nAChRs a promising therapeutic strategy. Galantamine modulates nAChRs to promote acetylcholine neurotransmission and also acts as an acetylcholinesterase (AChE) inhibitor. Galantamine was tested in preclinical experiments. Young and older rabbits received galantamine (3.0 mg/kg) for 15 days during conditioning, and the drug significantly improved learning, reduced AChE levels, and increased nAChR binding. In a second experiment, 53 retired breeder rabbits were tested over a 15-week period. A continuous dose of 3.0 mg/kg galantamine over 15 weeks ameliorated learning deficits significantly during acquisition and retention. NACHR binding was significantly increased in rabbits treated for 15 days with 3.0 mg/kg galantamine, and all galantamine-treated rabbits had lower levels of brain AChE. In a third experiment, galantamine reversed the effect of the nicotinic antagonist, mecamylamine. The efficacy of galantamine in a learning paradigm severely impaired in AD is consistent with outcomes in clinical studies.

XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY



Poster Abstracts



**This Page Intentionally
Left Blank**

EFFECT OF THYROID HORMONE ON ACETYLCHOLINESTERASE mRNA LEVELS IN THE SLOW SOLEUS AND FAST EDL MUSCLE OF THE RAT

P. Pregelj, J. Sketelj

*Institute of Pathophysiology, School of Medicine, University of Ljubljana,
Slovenia*

Acetylcholinesterase (AChE) is essential for normal synaptic transmission in the neuromuscular junction. In the rat, the level of AChE mRNA in the typical slow soleus muscle is only about 20-30% of that in the predominantly fast extensor digitorum longus (EDL) muscles. It is known that the expression of contractile proteins in muscles is influenced by hormonal milieu and that hyperthyroidism makes slow soleus muscle faster. The influence of thyroid hormone status on the levels of acetylcholinesterase (AChE) mRNA level in the slow antigravity soleus and fast EDL muscle of the rat was studied in order to examine the role of thyroid hormones in comparison to other factors such as muscle load, innervation and muscle activation pattern. Hyperthyroidism was induced in rats by daily thyroid hormone injection and hypothyroidism by thyroidectomy. A group with tonic electrical stimulation of sciatic nerve. Hindlimb suspension was used to produce muscle unloading. AChE mRNA levels were analyzed by Northern blots and evaluated densitometrically. We observed that AChE transcript levels increased in the soleus muscle of hyperthyreotic rats with or without hindlimb suspension, and decreased in hypothyreotic rats. However, increase of AChE mRNA was not observed in denervated soleus muscle of hyperthyreotic rats. On the other hand, a decrease of AChE mRNA levels in the EDL muscle observed in hindlimb suspended rats was not found in hyperthyreotic hindlimb suspended rats. These results corroborate the hypothesis that thyroid hormone status is an important independent extrinsic factor in AChE mRNA regulation in the slow soleus and fast EDL muscle.

NEUROMUSCULAR JUNCTION FORMED IN CO-CULTURE WITH EMBRYONIC SPINAL CORD IS ACCOMPANIED BY CO-DIFFERENTIATION OF NEURONAL AND GLIAL CELLS

T. Mars¹, K.J. Yu², X. Tang³, A.F. Miranda⁴, Z. Grubic¹, F. Cambi³,
M.P. King²

¹*Institute of Pathophysiology, School of Medicine, University of Ljubljana,
Slovenia,* ²*Department of Biochemistry and Molecular Pharmacology,
Thomas Jefferson University, Philadelphia, USA,* ³*Department of
Neurology, Thomas Jefferson University, Philadelphia, USA,* ⁴*Department
of Neurology, College of Physicians and Surgeons, Columbia University,
New York, USA*

Dissociated motor neurons, co-cultured with myotubes, form only immature neuromuscular contacts and usually die quickly. However, motor neurons originating from the explants of embryonic spinal cord form long lived and highly differentiated neuromuscular junctions (NMJ) with cultured muscle. In the light of well documented importance of glia in neuronal development, it can be hypothesized that this difference is due to the presence of glial cells in the spinal cord explants and that co-differentiation of neuronal and glial cells contributes to NMJ differentiation. Supporting this hypothesis necessitates the demonstration that such co-differentiation indeed proceeds in the system. No study addressing this question has been done yet. By immunocytochemical and biochemical analyses we followed the expression of stage-specific markers of neuronal and glial differentiation in co-cultures of human muscle and explants of embryonic rat spinal cord and found that the maturation of the neuromuscular junctions, motor neurons and astrocytes, oligodendrocytes and Schwann cells follows almost the same temporal pattern as observed *in vivo*. These findings strongly support the view that formation of differentiated and long-lived neuromuscular junction involves interactions among neuronal and glial cells. Adequate precaution is therefore necessary at the interpretation of results in various *in vitro* models employed to study synaptogenesis of NMJ.

ACETYLCHOLINESTERASE mRNA EXPRESSION IN RAT SPINAL CORD

K. Mis¹, E. Davidson², H. Park², M.P. King², T. Mars¹, Z. Grubic¹

¹*Institute of Pathophysiology, School of Medicine, University of Ljubljana,
Slovenia,* ²*Department of Biochemistry and Molecular Pharmacology,
Thomas Jefferson University, Philadelphia, PA, USA*

The function of acetylcholinesterase (AChE) in the CNS is not as well defined as in the neuromuscular junction (NMJ). Besides terminating the cholinergic inputs to motor and other neurons, high concentrations of acetylcholinesterase (AChE) in the spinal cord might also reflect other cholinergic and non-cholinergic roles of this molecule. Transportation of the asymmetric A12 form along axons of motor neurons suggests that AChE synthesized in motor neurons also contributes to the pool of basal lamina-bound AChE in the NMJ. To elucidate the role of AChE in the spinal cord we studied the expression of AChE in this part of the CNS. By combining *in situ* hybridization with fluorescent nuclear labeling and choline acetyltransferase staining, we found that glial cells are AChE mRNA negative and that motor neurons are the only cells expressing this mRNA in the ventral horns of the rat spinal cord. Further, we quantitated by competitive RT-PCR the expression of AChE mRNA in the spinal cord between E15 and E21 of rat embryonic development when the NMJs are formed. We found that expression of AChE mRNA follows the same temporal pattern of expression as mRNA encoding the neuronal isoform of agrin (AGRI9), which is released from the motor nerve ending and is bound to the synaptic basal lamina in the NMJ. The same pattern of expression of AChE and AGRI9 in the motor nerve may be a reflection of common processing and targeting of these two molecules to the same location and therefore suggests that, at least during the initial stages of NMJ formation, a part of AChE in NMJ may be contributed by a motor neuron.

FUNCTIONAL EXPRESSION AND STOICHIOMETRY OF THE NOVEL HUMAN $\alpha 9\alpha 10$ HETEROMERIC NICOTINIC ACETYLCHOLINE RECEPTOR

C. Grantham, I. Vandenberk, D. Nieuwstraten, P. Groot-Kormelink,
L. Van der Helm, J. Yon, A. Kremer, P. Van der Spek, S. Masure,
W. Luyten, J. Andrews

Janssen Research Foundation, Beerse, Belgium

We have cloned a novel human neuronal nicotinic acetylcholine receptor (nAChR) subunit, termed $\alpha 10$ (Genbank accession number AJ295237). The $\alpha 10$ nucleotide sequence maps to chromosome 11 (p15.5) and the predicted $\alpha 10$ polypeptide translation product is 66% similar to that of the human $\alpha 9$ subunit. $\alpha 10$ alone does not form a functional nAChR in oocytes but when it is co-expressed with $\alpha 9$ oocytes responded to bath applied ACh (30 μ M) with much larger currents (1.07 \pm 1.34 μ A; N=27) compared to oocytes expressing only $\alpha 9$ (0.07 \pm 0.06 μ A; N=23). However, co-expression of $\alpha 10$ with $\alpha 9$ does not dramatically change the EC₅₀ value to ACh (10.3 μ M for $\alpha 9\alpha 10$ versus 14.4 μ M for $\alpha 9$ alone). To conclusively prove that $\alpha 10$ co-assembles with $\alpha 9$ to create a functional receptor we introduced a reporter mutation in $\alpha 9$ (L277T) and $\alpha 10$ (L276T) and determined EC₅₀ values for various $\alpha 9\alpha 10$ combinations. Mutating $\alpha 10$ shifted the ACh concentration-response curve to the left (EC₅₀=2.9 μ M; 95% confidence limits (CL) 2.5 to 3.3) while mutating $\alpha 9$ resulted in an intermediate sensitivity to ACh (EC₅₀=4.6 μ M; 95% CL 4.2 to 5.1). nAChRs composed only of mutant $\alpha 9$ were the most sensitive to ACh (EC₅₀=0.5 μ M; 95% CL 0.4 to 0.7). These observations confirm that the $\alpha 10$ subunit co-assembles with $\alpha 9$ to produce a functional heteromeric $\alpha 9\alpha 10$ nAChR. Furthermore, they suggest a pentameric receptor stoichiometry in which there are more $\alpha 10$ subunits than $\alpha 9$, most likely two $\alpha 9$ and three $\alpha 10$.

LARGE SCALE EXPRESSION OF THE EXTRACELLULAR AND CYTOPLASMIC DOMAINS OF THE DROSOPHILA ADHESION PROTEIN, GLIOTACTIN

A. Solomon¹, R. Marcion¹, D.M. Rees¹, E.H. Rydberg¹,
T. Zeev Ben-Mordechai¹, S. Botti¹, I. Silman², J.I. Sussman¹, V.J. Auld¹

¹Department of Structural Biology, ²Department of Neurology,
Weizmann Institute of Science, Rehovot, Israel

The transmembrane cell adhesion protein Gliotactin (Gli) from *Drosophila* (Auld et al, 1995), a member of the acetylcholinesterase (AChE) alpha/beta hydrolase superfamily, shares 24% sequence identity with *Torpedo californica* AChE (TcAChE), including two residues of the catalytic triad and two of three disulfide bonds. The extracellular portion is believed to share a common global fold with AChE, while the intracellular portion has no significant similarity to any protein in the sequence databases. This protein is therefore of interest for several reasons. Confirmation of a similar fold for Gli and AChE would have important implications for recently suggested non-classical functions of AChE. In addition, the intracellular portion of Gli is an excellent candidate for structural genomics. Therefore, the C-terminal, intracellular portion of Gli (Gli_ct) and the N-terminal, cholinesterase-like portion (Gli_nt), were cloned separately into pET21b. Gli_ct was expressed in soluble form in *E. coli* (BLR) at 7 mg/L and purified to 95% homogeneity using Ni-NTA chromatography followed by size exclusion chromatography. Small crystals have been obtained, and optimization of crystallization conditions is in progress. Gli_nt was also expressed in soluble form in *E. coli* (Origami(DE3)pLysS) at 1 mg/L, purification is currently underway.

Reference:

Auld, V.J., Fetter, R.D., Broadie, K., Goodman, C.S. (1995)
Cell 81:757-767.

COEXPRESSION OF ALPHA10 AND ALPHA9 NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT DORSAL ROOT GANGLION NEURONS

K.S. Lips, U. Pfeil, R.V. Haberberger, W. Kummer

Institute for Anatomy and Cell Biology, Justus-Liebig-University,
Giessen, Germany

Acetylcholine excites sensory neurons of dorsal root ganglia (DRG) via both muscarinic and nicotinic acetylcholine receptors (nAChR). Until recently, 9 different α and 4 different β subunits of nAChR that form pentamers to yield functionally active receptors have been identified. However, binding and immunoprecipitation studies revealed a population of nAChR in DRG that shall contain at least one additional, unknown subunit. This year, a new member of the nAChR family ($\alpha 10$) has been identified in cochlear hair cells. It forms heteromers with $\alpha 9$ that share a crucial feature with the unknown nAChR of DRG, i.e. sensitivity to α -bungarotoxin. Here, we asked whether these subtypes are also expressed in rat DRG, and addressed this issue by RT-PCR, in-situ hybridization, and using a newly raised antibody against the $\alpha 10$ subunit. All sensory neurons, but no glial cells, expressed both $\alpha 9$ and $\alpha 10$ mRNA, and exhibited $\alpha 10$ -immunoreactivity. These in-situ hybridization and immunohistochemical findings were confirmed by RT-PCR. These data show that coexpression of $\alpha 9$ and $\alpha 10$ nAChR subunits is not restricted to hair cells but occurs also in DRG sensory neurons where it is likely to represent the predicted, previously unknown nAChR. (supported by the DFG, SFB 547).

STRUCTURAL REORGANIZATION OF THE ACETYLCHOLINE BINDING SITE OF TORPEDO NICOTINIC RECEPTOR REVEALED BY DYNAMIC PHOTOAFFINITY LABELING

T. Grutter¹, F. Kotzyba-Hibert², S. Bertrand³, D. Bertrand³, M. Goeldner²

¹Institut Pasteur, Paris, France, ²Faculté de Pharmacie, Illkirch, France.

³Faculté de Médecine, Geneva, Switzerland

We have explored by photoaffinity labeling the structural changes that occurred at the acetylcholine (ACh) binding site of the *Torpedo* nicotinic receptor (nAChR) during activation by a tritiated photoactivatable agonist [³H]DCTA(diazocyclohexadienylpropyl)-trimethylammonium). The agonistic nature of DCTA was confirmed both at the muscular and neuronal nicotinic receptors reconstituted in *Xenopus* oocytes. In a previous study, the identification of labeled residues by [³H]DCTA allowed to characterize the amino acids involved in the binding of the ester moiety of ACh in the desensitized state [1]. In the present work, the agonist character of DCTA was used to label functional states.

A stopped-flow apparatus was adapted for dynamic photolabeling. After selected mixing times between nAChR and [³H]DCTA in a delay loop, samples were irradiated by intense UV light preceding the collection for biochemical analysis. We quantified a saturable increase of specific photolabeling on the alpha and gamma subunits. We further analyzed this incorporation either after rapid mixing (500 ms) or after equilibration (50 min) of [³H]DCTA with nAChR; hence the probe explores transient state(s) and the stable desensitized state, respectively. Comparative analysis showed a differential photoincorporation of [³H]DCTA and suggests that in the course of agonist-induced desensitization of the receptor, the site lining peptide loops from adjacent alpha and gamma subunits move closer together.

[1] Grutter, T. et al. (2000) *Biochemistry* 39, 3034-3043

MAPPING THE ACETYLCHOLINE BINDING SITES OF TORPEDO NICOTINIC RECEPTOR USING PHOTOAFFINITY LABELING: PAST, PRESENT AND FUTURE

F. Kotzyba-Hibert, A. Mourou, T. Grutter, M. Goeldner

Université Louis Pasteur, Faculté de Pharmacie, Illkirch, France

Topographical mapping of residues contributing to the ACh binding sites was achieved with site-directed antagonists or agonists labels. From these studies and mutagenesis three discontinuous domains of the alpha-subunit (loops A, B and C) with additional residues on the gamma and delta subunits (loops D, E and F) were identified (1).

The structural reorganization that occurs at the cholinergic binding domains on desensitization was studied at the molecular level first with [³H]DDF using flash photolysis coupled to a stopped flow apparatus and more recently with the agonist [³H]DCTA. [³H]DCTA was able for the first time to explore, at the molecular level, transient states of nAChR upon agonist activation showing a modified labeling pattern for amino acids labeled in loop C and for gamma and delta fragment's peptides (see poster T. Grutter et al.).

To investigate more precisely the gamma (or delta) peptides determinants involved in the structural rearrangement upon agonist activation, we used a formerly described photosensitive agonist [³H]AC5 whose labeling pattern on *Torpedo* nAChR ACh binding area involved all four subunits (2). This pattern was modified upon receptor desensitization according to preliminary dynamic photolabeling experiments.

Identification of gamma and delta residues involved in subunits reorganization around the binding sites and modelisation of nAChR *Torpedo* at the alpha-gamma and alpha-delta interfaces based on the X-ray structure of AChBP will give new insights in allosteric transitions upon desensitization.

[1] P.-J. Corringer et al. (2000) *Ann. Rev. Pharmacol. Toxicol.* 40, 431-458

[2] B. Chairenet et al. (1992) *Mol. Pharmacol.* 41, 1100-1106.

NFKB REGULATES THE ACTIVITY OF HUMAN ACETYLCHOLINESTERASE PROMOTER IN MUSCLE

Roy C.Y. Choi¹, Nina L. Siow¹, Anthony W.M. Cheng¹,
David C.C. Wan², Karl W.K. Tsim¹

¹Department of Biology and Molecular Neuroscience Center, The Hong Kong University of Science and Technology, Hong Kong, China.

²Department of Biochemistry, The Chinese University of Hong Kong, China

Precise expression of biological active acetylcholinesterase (AChE; EC 3.1.1.7) is required for development and maintenance of the neuromuscular junctions. Previous studies reveal that the expression of human AChE in muscle can be regulated by the classical cAMP-dependent pathway that involved cAMP-dependent protein kinase (PKA) and cAMP-responsive element binding protein (CREB), via the regulatory element called cAMP-responsive element (CRE) on promoter of the enzyme. In the present study, we investigated the role of another regulatory element namely NFkB, in regulating the AChE promoter activity. A 2.2 kb human AChE promoter was tagged with a luciferase reporter gene, and the construct was transfected in chick myotube cultures for analyzing the luciferase activity. The transcriptional activity of human AChE promoter was shown to be regulated by NFkB signaling. One of the possible nerve-derived factors that utilizes this mechanism is the adenosine 5-triphosphate (ATP) and its correspondent receptor called P2Y purinoceptor. All these data suggested that the AChE promoter could be regulated by multiple signaling pathways for precise transcriptional control at the post-synaptic muscle.

Acknowledgments:

supported by grants from the Research Grants Council of Hong Kong (HKUST 6099/98M, 6112/00M & 2/99C).

ROLE OF MDX NERVE AND MUSCLE IN REGULATING NEUROMUSCULAR JUNCTION PROPERTIES: A STUDY USING MUSCLE TRANSPLANTS

A.R. Durrant¹, M. Szabo¹, L.L. Anglister², M.M. Salpeter¹

¹Cornell University, Ithaca, NY, USA, ²Hebrew University, Hadassah Medical School, Jerusalem, Israel

Neuromuscular junctions (NMJs) of mdx (X-linked dystrophy) mutant mice have fewer junctional folds, altered shape, lower AChR density, and accelerated AChR degradation rate. The relative contributions of nerve and muscle to this organization are unknown. The sternomastoid muscle of mdx and wild-type mice were surgically exchanged into host counterparts, and 7.5 mo. later examined for expression of dystrophin protein and NMJ fine-structure. The dystrophin staining patterns were maintained in the transplants, and when the wild-type was the host, the transplanted mdx muscle exhibited extensive recovery of junctional folds. To analyze the NMJ shape, we labeled AChRs in 4.5 mo. transplants with fluorescent alpha-bungarotoxin, Bgt. Labeled AChRs were redistributed at the NMJ; when the wild-type was the host, the shape of NMJ in the donor mdx muscle was more contiguous than when the mdx was the host. To determine if AChR number was affected by host phenotype, we labeled AChRs in 7.5 mo. transplants by injecting 125I-Bgt. Two days later, the donor muscle was assessed for AChR radioactivity using a gamma counter. Consistent with the change in junctional folds and NMJ shape, the mdx transplant into wild-type host showed 125I-Bgt binding similar to control wild-type muscles, while that of the wild-type transplant into mdx host was comparable to control mdx muscles. Together, these findings suggest that the donor genotype of the muscle is maintained and that the host (and nerve) influences NMJ fold formation, shape, and total number of AChRs.

Supported by NIH NS 09315 (MS) and NIH GM 07469 (AD).

THE INITIAL BINDING OF ACETYLCHOLINESTERASE AND PERLECAN OCCURS INSIDE THE CELL PRIOR TO EXTERNALIZATION

S.G. Rossi, R.L. Rotundo

Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, Florida, USA

The collagen-tailed form (A12) of acetylcholinesterase (AChE) is attached to the extracellular matrix on the surface of myotubes in culture and on the basal lamina at sites of nerve-muscle contact "in vivo". This basal lamina-associated AChE is tightly linked to the extracellular matrix through the heparan sulfate proteoglycan perlecan. Where and how this linkage occurs is not known. One possibility is that the initial interaction between AChE and perlecan occurs post-translationally during intracellular trafficking. To test this hypothesis, we incubated quail muscle culture extracts with protein-A Sepharose beads bound with anti-perlecan monoclonal antibody and assayed them for bound AChE. Anti-perlecan antibodies immunoprecipitated about 5-10 % of total AChE activity. To determine whether this binding occurred intracellularly, muscle cultures were treated with DFP, an irreversible AChE inhibitor, followed by incubation in complete medium to allow de-novo synthesis of AChE. This time course study showed that AChE binding to perlecan occurred approximately 2-3 hours after synthesis, coincident with the assembly of A12 AChE in the Golgi apparatus. At this time A12 AChE is approximately 15 % of the total enzyme synthesized, and about the same percentage of the newly synthesized intracellular AChE pool binds to anti-perlecan beads. We concluded that the newly synthesized A12 AChE is assembled with perlecan inside the cell prior to externalization.

CRYSTAL STRUCTURE OF THE TETRAMERIZATION DOMAIN OF ACETYLCHOLINESTERASE AT 2.3A RESOLUTION

M. Harel¹, H. Dvir¹, S. Bon², W.Q. Liu³, C. Garbay³, J.L. Sussman¹,
J. Massoulié², I. Silman¹

¹Dept of Structural Biology and Neurobiology, Weizmann Institute of Science, Rehovot, Israel, ²Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France, ³Pharmacochimie Moléculaire et Structurale, Paris, France

Tetramerization of acetylcholinesterase (AChE) is achieved by the interaction of 2 peptide motifs: a 40-residue 'tryptophan amphiphilic tetramerization' (WAT), at the C-terminus of the catalytic subunit, and a 17-residue 'proline-rich attachment domain' (PRAD), localized near the N-terminus of the ColQ collagenic tail polypeptide, with 4:1 WAT:PRAD stoichiometry. The two peptides were produced by chemical synthesis. Met21 of WAT was replaced by selenomethionine, to facilitate a MAD diffraction experiment. The synthetic WAT and PRAD were mixed at a 4:1 ratio, and co-crystallized. The monoclinic crystals obtained diffracted to 2.3A resolution, and MAD data sets were collected at the synchrotron. The structure was solved with the program SOLVE which produced a traceable electron density map. The structure was refined to an R-factor of 24.6% with the 2 PRADs seen in full and the 8 WATs having disordered C-termini. The WAT chains assume an α -helical conformation, and are all parallel. The PRAD has a polyproline II conformation and threads its way anti-parallel to the WAT chains. Most of the 3 highly conserved Trp residues in each WAT chain are stacked against the 8 Pro residues or 3 Phe residues of the single PRAD. An AChE tetramer structure can be modeled based on the structure of the WAT/PRAD complex.

SCALING UP OF PRODUCTION, PURIFICATION AND REFOLDING OF A CHIMERIC THREE-FINGERED TOXIN WITH SPECIFICITY FOR ACETYLCHOLINESTERASE

D. Shaya¹, F. Ducancel², M.H. Le Du², Alejandro Ricciardi³, A. Ménez², I. Silman¹, J. L. Sussman¹

Departments of Structural Biology¹ and Neurobiology¹, Weizmann Institute of Science, Israel, ²Département d'Ingénierie et d'Etude des Protéines, Commissariat à l'Energie Atomique, CE Saclay, Gif-Sur-Yvette Cedex, France, ³Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay

Chimera II (rchII) is an engineered hybrid toxin constructed from two members of the "Three-Finger-Toxins" family: fasciculin 2 from the green mamba, *Dendroaspis angusticeps*, a potent inhibitor of acetylcholinesterase (AChE), and toxin α from the black neck spitting cobra, *Naja nigricollis*, a potent inhibitor of the nicotinic acetylcholine receptor. The 3D structures of both toxins are known and reveal a striking resemblance in their cores with significant difference in their fingers. The structure of the fasciculin 2/AChE complex has also been solved.

The 3D structure of rchII consists of the globular core of toxin α (3 anti-parallel β -strands) together with 2 of the extended loops (fingers) connecting these strands from fasciculin 2. rchII inhibits *E. electricus* AChE with an affinity that is 15-fold less than native fasciculin 2. The chimera was expressed, purified, crystallized and its structure solved (Ricciardi *et al.* *J. Biol. Chem.* [2000] **275**, 18302-18310; Le Du *et al.* *J. Mol. Biol.* [2000] **296**, 1017-1026).

Scaling up of production of rchII was initiated to produce amounts adequate for determination of the structure of rchII/Torpedo californica AChE (TcAChE) complex and for more detailed kinetic and physicochemical studies. It included large-scale production of bacterial host cells (10 L), to yield 150 mg of fusion protein per liter of bacterial culture. Additional HPLC steps were added in the purification of the unfolded chimeric polypeptide to yield 10 mg of unfolded polypeptide per liter of initial bacterial culture, which were then taken for the refolding step.

EXPRESSION OF COLQ AT THE NEUROMUSCULAR JUNCTION

C. Legay

Ecole Normale Supérieure, Paris, France

ColQ, a specific collagen, has been identified in Torpedo and mammals (Krejci *et al.*, 1991, 1997). So far the main function attributed to this collagen is to anchor acetylcholinesterase (AChE) in the synaptic basal lamina at the neuromuscular junction (NMJ). Our data obtained by RT-PCR and in situ hybridisation show that the ColQ gene is produced both by the motoneurone and the muscle cell. However several variants of ColQ have been identified. On-going experiments are determining the structure of ColQ produced by the motoneurone. Is ColQ secreted in extrasynaptic domains where AChE is low? Studies from ColQ^{-/-} muscle cells in culture transfected with a GFP-ColQ cDNA suggest that ColQ can be secreted in the absence of AChE. We also transfected these cells with mutants of the ColQ heparin binding sites (HBS) or mutants of ColQ C-terminus and show that in both cases no spontaneous clusters of AChE can be detected, suggesting a complex mode of AChE anchoring in the synaptic basal lamina.

ROLE OF SPONTANEOUS MUTATIONS OF NEURONAL NICOTINIC RECEPTORS IN ADNFLE

D. Bertrand¹, I. Favre¹, H. Phillips², S. Bertrand¹, S.F. Berkovic³, J.C. Mulley²

¹Department of Physiology, Medical Faculty, Geneva, Switzerland.

²Department of Molecular Genetics, Women's and Children's Hospital, North Adelaide, Australia, ³Department of Medicine, Austin and Repatriation Medical Centre, Heidelberg, Melbourne, Australia

The association between a mutation in either the $\alpha 4$ or $\beta 2$ coding genes and a form of nocturnal epilepsy (ADNFLE) suggests that alteration of the nicotinic acetylcholine receptor can be at the origin of brain seizures. Thus, three spontaneous mutations in the $\alpha 4$ (S248F, 776ins3, S252L; Steinlein *et al.*, 1997) and two different substitutions of valine 287 in the $\beta 2$ subunit (V287L, V287M; De Fusco *et al.*, 2000; Phillips *et al.*, 2001) have been identified. These mutations modify amino acids within or near the second transmembrane domain of the protein (TM2). To identify the effects caused by these five mutations on the functional nicotinic receptor properties, exogenous expression of these proteins were designed. Determination of a series of physiological and pharmacological characteristics revealed that the single common trait identified so far is an increased sensitivity of the receptors to their endogenous ligand acetylcholine. Based on our understanding of the receptor distribution in the different brain areas, their development and the neuronal network circuitry, we hypothesize that an increased of the acetylcholine sensitivity may cause an unbalance in the fine-tuning of the cortico-reticular thalamic and thalamo-cortical network in favour of the latter. The use of specific agonists or antagonists of the $\alpha 4\beta 2$ receptor should help to elucidate the last steps in these critical brain pathways and therefore shine a new light on the fundamental mechanism of epilepsy.

This work was supported by the Swiss National Science Foundation to DB.

INTERACTION OF RECOMBINANT SOLUBLE NEUROLIGINS-1 WITH NEUREXIN-BETA

D. Comoletti¹, R. Flynn¹, L. Jennings¹, R. Hoffman², T. Matsumura¹, H. Hasegawa¹, P. Marchot³, Y. Bourne⁴, E. Komives⁵, T. Sodhof⁶, P. Taylor¹

¹Department of Pharmacology, University of California, San Diego, La Jolla, CA, USA, ²Howard Hughes Medical Institute Mass Spectrometry Facility, University of California, San Diego, La Jolla, CA, USA, ³Ingénierie des Protéines, CNRS UMR-6560, Institut Federatif de Recherche Jean Roche, Université de La Méditerranée, Marseille, France, ⁴Architecture et Fonction des Macromolécules Biologiques, CNRS UMR-6098, Marseille, France, ⁵Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA, USA, ⁶Department of Molecular Genetics, University of Texas Southwestern, Dallas, TX, USA

Neuroligins are postsynaptic transmembrane proteins of the α/β -hydrolase fold family with high sequence identity to the cholinesterases. They form heterologous cell contacts with the neuexins. To examine the neuroligin-1 (NL1) interactions with neuexin-1-beta (NX1beta), and to define the NL1 binding surface interacting with NX1beta, we expressed soluble exported recombinant forms of NL1 by truncating the carboxyl-terminal end prior to the transmembrane span at positions D638 and D691. LCMS of peptidic digests showed the pairing of the disulfide bonds and the carbohydrate composition of N- and O-linked glycosylation sites. NL1 truncated at D691 was crystallized by vapor diffusion and data up to 4.35 Å were collected on the ESRF beamline ID29. Attempts to solve the structure by molecular replacement were unsuccessful but efforts to solve a structure of a truncated NL1 devoid of O-glycans are underway. By surface plasmon resonance, we studied the association of purified NL1s with NX1beta. Elimination of the NL1 transmembrane span yielded soluble protein that associates with NX1beta with a KD in the nM range, while the homologous protein AChE does not bind NX1beta. Purified soluble NL1 binds two splice forms of NX1beta with slight differences in affinity. Incomplete post-translational processing of NL1 and enzymatic removal of certain oligosaccharides or sialic acids enhance activity, while enzymatic deglycosylation of neuexin-beta does not alter binding, indicating that glycosylation of NL1 may provide a further level of control in the neuexin-neuroligin association. Work supported by USPHS Grant GM-18360 to P.T.

CLONING AND CHARACTERIZATION OF ACETYLCHOLINESTERASE GENE IN CHICKEN

X. Zhang¹, K.W.K. Tsim², D.C.C. Wan¹

¹Department of Biochemistry, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China. ²Department of Biology, The Hong Kong University of Science and Technology, Hong Kong, China

Acetylcholinesterase (AChE) is a highly polymorphic enzyme with multiple forms and its regulation is under strict regulation. To understand more about the species-specific regulation of AChE gene in avian versus mammalian species, we have attempted to clone acetylcholinesterase gene in chicken by rapid amplification of genomic end (RAGE). Antisense primers complementary to the 5-prime coding region of the chicken AChE cDNA were used as nested PCR primers, which resulted in the isolation of the largest genomic fragment of about 2.1 kb. This genomic DNA contained a 269 bp sequence at the 3-prime end corresponding to 19-380 bp of the published chicken AChE cDNA with minor sequence discrepancy. The cDNA at position 90CGATTGCC98 was found to be CCGCATTTCCGCC, resulting in a change of deduced amino sequences from ArgPheAla to ProAspPheAla. TATAA box was found upstream the start codon and a BLAST search of the sequence immediately upstream to the start codon, that may confer the functional promoter activity, revealed little homology to the published AChE genes. The putative promoter fragment was subcloned into pGL3 basic vector for the luciferase promoter-reporter assay. Deletion analysis with a truncated 366 bp fragment upstream to ATG resulted in a ten-fold increase of the activity. PMA, forskolin and cAMP can induce the luciferase activity increase 60% to 150% in NG108-15 cells. Similar response to the drugs was also found in the chick myotubes. Further characterization of chick AChE promoter is under investigation. *Acknowledgments:* supported by a grant from the Research Grants Council of Hong Kong to DCW (CUHK 414/98M).

MUSCARINIC RECEPTOR REGULATION OF EVOKED ACETYLCHOLINE RELEASE IS AFFECTED BY ACETYLCHOLINESTERASE INACTIVATION AT THE MOUSE NEUROMUSCULAR JUNCTION

¹Jasmina Minic, ¹Jordi Molgó, ²Eric Krejci

¹UPR9040, CNRS, Gif Sur Yvette, ²UMR8544, ENS-CNRS, Paris, France

In the present work we used antibodies and Western blotting of lysates obtained from innervated and non-innervated regions of the mouse diaphragm to demonstrate that mouse neuromuscular junctions (NMJ) express mAChR subtypes M₁ through M₄, and that localization of all subtypes is restricted to the innervated part of the muscle. To elucidate the roles of the mAChR subtypes in regulating ACh release evoked by the presynaptic action potential, we evaluated the quantal content of endplate potentials by the method of failures on isolated phrenic-hemidiaphragm preparations removed from normal and collagen Q-deficient mice.

Muscarine reduced evoked ACh release in normal NMJs, but it enhanced ACh release both in collagen Q-deficient NMJs lacking acetylcholinesterase (AChE) and in normal junctions when AChE was inhibited by fasciculon-2. The muscarine-elicited depression of ACh release in normal NMJs was completely abolished by pre-treatment with PTX or methoctramine, a selective M₂ antagonist, but was not affected by M₄ selective muscarinic toxin MT-3. These results indicate the involvement of the M₂ mAChR in decreasing ACh release. The muscarine-induced increase of ACh release in AChE-deficient NMJs was not affected by PTX, but was completely blocked by a specific M₁ antagonist MT-7. Our results show that the M₁ and M₂ mAChRs have opposite presynaptic functions in modulating ACh release, and that regulation of transmitter release by the two receptor subtypes depends on the functional state of AChE at the NMJ.

CANDOXIN A NEW SNAKE TOXIN SPECIFIC FOR THE ALPHA7 nAChR

E. Charpantier¹, S. Nirthanan², P. Gopalakrishnakone², M.C.E. Gwee², H.E. Khoo², L.S. Cheah², R. Manjunatha Kini³, D. Bertrand¹

¹Department of Physiology, CMU, Geneva, Switzerland. ²Venom and Toxin Research Program, Faculty of Medicine, National University of Singapore. ³Department of Biological Sciences, Faculty of Science, National University of Singapore

Toxins isolated from animals provide important pharmacological tools for studies of ligand-gated ion channels. Snake toxins like alpha-bungarotoxin are known to inhibit almost irreversibly the muscle nicotinic acetylcholine receptor (nAChRs). In addition, alpha-bungarotoxin was shown to powerfully block the alpha7 neuronal nAChRs. Snake toxins are divided in short and long-chain according to the number of amino acids they contain. A further classification is often made in function of their number of disulfide bridges. The different snake toxins share the same structural form, constituted by three loops (I, II and III) protruding from a globular core whereas the loop II contains the principal amino acids participating to the nAChRs recognition. Candoxin is a new toxin that was isolated from the Malayan krait *Bungarus candidus*. Constituted by 66 residues and displaying the typical three fingers motif with five disulfide bridges, candoxin shares 30% homologies with the alpha-bungarotoxin. Candoxin is a member of the short chain toxins and is closely related to neurotoxins (98% homologies) that were isolated from *Bungarus multicinctus*. When tested on heterologously expressed receptors, candoxin was found to strongly antagonize either the muscle or the alpha7 rat nAChRs. The main difference with other known toxin is, however, the almost irreversible blockade of candoxin at the neuronal alpha7 receptors, while the muscle receptors readily recovered from blockade within minutes. Therefore, candoxin provides a new tool for the studies of the neuronal nAChRs in the central nervous system.

E.COLI EXPRESSED EXTRACELLULAR DOMAIN OF RAT ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR: PHYSICO-CHEMICAL AND BINDING PROPERTIES

N.I. Dergousova, E.A. Azeeva, E.V. Kryukova, E.D. Shibanova, I.E. Kasheverov, A.S. Korotina, Y.N. Utkin, V.I. Tsetlin

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia

The title domain (amino acid residues 1-208) was expressed as a fusion protein with glutathione S-transferase (GST). Refolding of GST-(1-208) protein in the presence of 0.1% SDS and the C116S mutation considerably decreased the formation of high-molecular weight aggregates. Gel-permeation HPLC was used to isolate the monomeric forms of the GST-(1-208) and its mutant almost devoid of SDS. CD spectra revealed that the mutation considerably increased the content of beta-structure and made it more stable at different conditions. The proteins obtained bound iodinated alpha-bungarotoxin with K_d about 100 nM, the C116S mutation slightly increasing the affinity for the toxin. The proteins retained such selectivity features of the intact alpha7 as discrimination of long-chain alpha-neurotoxins and alpha-conotoxin ImI against short-chain neurotoxins and conotoxin G11. The alpha7 domain devoid of the fusion moiety was found to bind biotinylated alpha-cobratoxin which could be displaced by alpha-cobratoxin itself, d-tubocurarine or anabaseine. Therefore, the obtained proteins seem promising for further structural analysis and for screening purposes as demonstrated recently (Utkin et al. 2001) by unraveling the alpha7 binding capacity in the so-called weak toxin.

NICOTINIC ACETYLCHOLINE RECEPTOR AND MUSK ARE CLUSTERED IN C2C12 CELLS VIA LIPIDIC RAFTS

F. Stetzkowski-Marden, S. Marchand, J. Cartaud

Institut Jacques Monod, University Paris 7, France

Clustering of nicotinic acetylcholine receptors (AChRs) and signaling molecules such as the Muscle-specific Receptor Tyrosine Kinase MuSK in the postsynaptic membrane is the landmark of synaptic differentiation at the neuromuscular junction. Cholesterol-sphingolipid-enriched microdomains or rafts are part of the cell machinery ensuring correct intracellular trafficking of selected proteins and lipids, and have been shown to participate in the formation and maintenance of alpha-7 nAChR clusters in somatic spines of ciliary neurons (Brusés et al., 2001). In addition, cells defective in sphingolipids biosynthesis were shown to express low amounts of muscle AChR, suggesting that sphingolipid metabolism may influence trafficking of the protein to the surface membrane (Roccamo et al., 1999). In this work, we have asked whether rafts are engaged in AChR clustering in muscle cells. Using filipin, a fluorescent marker of cholesterol, and beta-cyclodextrin, a drug that deplete cells from cholesterol, we showed that cholesterol is present within agrin-induced AChR clusters in C2C12 cells and is necessary to maintain these clusters. Non-ionic detergent extraction on ice and flotation gradient centrifugation of COS7 cells transfected with AChR subunits cDNAs demonstrate that AChRs are contained in low density (5-30% interface) fractions enriched in two rafts markers, flotillin and caveolin. Yet, in C2C12 myotubes, AChRs and MuSK are recovered in the 30% sucrose fraction following solubilization with Lubrol. We conclude that AChR as well as important signaling molecules such as MuSK are accumulated at postsynaptic sites via their association with large raft-like microdomains.

ACETYLCHOLINESTERASE IS REQUIRED FOR NEURONAL AND MUSCULAR DEVELOPMENT IN ZEBRAFISH

M. Behra¹, X. Cousin², C. Bertrand², J.-L. Vonesch¹, A. Chatonnet², U. Strohle¹

¹IGBMC, Illkirch, ²INRA, Montpellier, France

The neurotransmitter acetylcholine (ACh) has a crucial role in central and neuromuscular synapses of the cholinergic system. After release into the synaptic cleft, ACh is rapidly degraded by acetylcholinesterase (AChE). We have identified a mutation in the ache gene of the zebrafish, which abolishes ACh hydrolysis in homozygous animals completely. Embryos are initially motile but subsequently develop paralysis. Mutant embryos show defects in muscle fiber formation and innervation, and primary sensory neurons die prematurely. The neuromuscular phenotype in ache mutants is suppressed by a homozygous loss-of-function allele of the alpha-subunit of the nicotinic acetylcholine receptor (nAChR), indicating that the impairment of neuromuscular development is mediated by activation of nAChR in the mutant. Here we provide genetic evidence for non-classical functions of AChE in vertebrate development.

Published in Nature Neuroscience, online: 2 January 2002.

IDENTIFICATION OF SPECIES DIFFERENCES IN THE PHARMACOLOGY OF THE ALPHA-7 NICOTINIC RECEPTOR USING THE ANTAGONIST RADIOLIGAND [3H]-METHYLLYCAONITINE

N. Crawford, K. Finlayson, J. Sharkey, J.S. Kelly

Fujisawa Institute of Neuroscience, Department of Neuroscience, University of Edinburgh, UK

It has been suggested that the impairment in cholinergic transmission observed early in Alzheimer's disease (AD) may partly underlie the cognitive decline associated with the condition. Recent reports that the alpha-7 nicotinic receptor (a7nAChR) co-localises with amyloid plaques and serves as a high affinity binding site for the beta-amyloid peptide, AB1-42, suggests this receptor may play a role in AD pathophysiology (Wang et al., 2000). Membranes were prepared from a cell line expressing human a7nAChRs (SH-EPI-ha7; Peng et al., 1999), or rat brain tissue, and binding assays performed using the selective a7nAChR receptor antagonist [3H]-methyllycaconitine ([3H]-MLA; Davis et al., 2000). A range of compounds were used to examine the pharmacology of [3H]-MLA binding sites, and for rat forebrain the rank order of potency was; MLA>(-)ARR-17779>E2020>tacrine=(+)ARR-17779>RJR2403=mecamylamine. When membranes expressing the ha7 receptor were used, antagonist affinities and that of the acetylcholinesterase inhibitors were similar to rat. In contrast, the affinities of the agonists for ha7nAChRs were approximately 100-fold higher when compared to rat tissue. Furthermore, under the conditions used in the present study, beta-amyloid had no effect on [3H]-MLA binding to human or rat a7nAChRs. Preliminary studies indicate [3H]-MLA binding to mouse membranes is consistent with rat data. In conclusion, we have demonstrated species differences in the pharmacology of [3H]-MLA binding sites, however, the lack of effect of beta-amyloid at both human and rat binding sites requires further clarification. Wang et al. (2000) J.Biol.Chem. 275(8) p5626. Peng et al., (1999) Brain Res. 825 p172. Davis et al. (2000) Eur.J.Neurosci. 12: p374.

THE 14.3.3 GAMMA PROTEIN IS PART OF THE MUSK SIGNALING COMPLEX AT THE COMPLEX AT THE NEUROMUSCULAR JUNCTION

L. Strohlic¹, A. Cartaud¹, M. Recouvreur¹, R. Grailhe², J.-P. Changeux², J. Cartaud¹

¹Institut Jacques Monod CNRS/Universities Paris VI et VII Paris,

²Institut Pasteur CNRS, Paris, France

The Muscle-Specific receptor tyrosine Kinase, MuSK, forms a receptor complex activated by nerve-derived agrin that orchestrates the differentiation of the neuromuscular junction (NMJ). To identify partners and/or effectors of MuSK, crosslinking and immunopurification experiments have been performed in purified postsynaptic membranes from Torpedo electrocytes. In a first series of experiments, this approach has lead to the identification of a PDZ-containing protein MAGI-1c (Strohlic et al. J.Cell Biol., 153: 1127-1132, 2001) In this work, a second polypeptide corresponding to the 14.3.3 gamma protein was identified by MALDI-TOF mass spectrometry (MS) analysis of the MuSK cross-link products. Moreover, MuSK and 14.3.3 gamma protein colocalized at the rat NMJ and co-immunoprecipitated following transient transfection in COS7 cells, suggesting a direct interaction between these two proteins. Transfections experiments in C2C12 myotubes show that the 14.3.3 gamma protein downregulates the transcription of the epsilon subunit of the AChR activated by ARIA/neuregulin1. Members of the 14.3.3 family of proteins play a central role in the regulation of various signaling pathways. Our data indicate that in addition to its role in AChR aggregation, the MuSK complex may be involved in the regulation of AChR transcription at the NMJ, possibly via the regulation of the MAP Kinase pathway by the 14.3.3 gamma protein.

CONSTRUCTION AND CHARACTERISATION OF A CHIMERIC HUMAN ALPHA 7 NICOTINIC ACETYLCHOLINE / MOUSE 5HT3 RECEPTOR

P.J. Craig¹, R. Zwart¹, S. Bose¹, R.E. Beattie¹, E.A. Folly¹, L.R. Johnson¹, E. Bell¹, N.M. Evans¹, S.G. Volsen¹, E. Sher¹, N.S. Millar², L.M. Broad¹

¹Lilly Research Centre, Windlesham, ²University College London, UK

The neuronal nicotinic acetylcholine receptor alpha 7 subunit can assemble as a homo-pentamer to form a functional ligand-gated ion channel. Each subunit comprises four membrane-spanning regions linked by alternate cytoplasmic and extracellular loops, together with extracellular N- and C-terminal domains. Chimeric alpha 7 / 5HT3 constructs have been described previously as tools for investigating the role of the various domains of the receptor. We report on the construction and characterisation of a novel human alpha 7 / mouse 5HT3 chimera. A chimera was constructed comprising the N-terminal region of the human alpha 7 nicotinic acetylcholine receptor linked at valine 202 with the transmembrane / C-terminal regions of the mouse 5HT3 receptor. Expression in *Xenopus* oocytes or HEK-293 cells resulted in functional channels that were sensitive to ligands of nicotinic acetylcholine, but not 5HT3 receptors. Currents obtained from oocytes injected with cDNA for the chimera desensitised more slowly than those obtained by injection of wild-type alpha 7. The response of both wild-type and chimeric receptors was potentiated by 5OH-indol. Expression in mammalian cells was initially demonstrated by surface alpha-bungarotoxin binding and single-cell calcium imaging in transiently transfected HEK-293 cells. Subsequently, stable clones were produced and functional clones selected by assessing agonist induced calcium increase in a Fura-2. The best responding clones were used to characterise the chimera pharmacologically using standard nAChR ligands.

ALPHA-CONOTOXINS PnIA AND A10L-PnIA STABILISE DIFFERENT STATES OF THE CHICK NEURONAL ALPHA 7 ACETYLCHOLINE RECEPTOR

R.C. Hogg¹, S. Bertrand², P.F. Alewood¹, D.J. Adams¹, D.C. Bertrand²

¹School of Biomedical Sciences, University of Queensland, Brisbane, Australia, ²Department of Physiology, CMU, Geneva, Switzerland

The alpha-conotoxins PnIA and the single amino acid substitution [A10L]PnIA are inhibitors of neuronal nicotinic acetylcholine receptors (nAChRs) in dissociated neurones (1) and recombinant alpha7 receptors expressed in *Xenopus* oocytes. The effects of PnIA, [A10L]PnIA and alanine scan mutants of [A10L]PnIA were investigated in homomeric chick alpha7 nAChRs and alpha7 receptors using the L247T mutant (2). PnIA inhibited the ACh-evoked current in the wild type alpha7 receptor and the alpha7 L247T mutant in a similar manner with IC50 values of 349 and 194 nM, respectively. All [A10L]PnIA alanine mutants investigated inhibited the ACh-activated current in wild type alpha7 nAChRs. In contrast, when co-applied with ACh, [A10L]PnIA and the alanine scan mutants all potentiated alpha7 L247T responses and in addition [A10L]PnIA was able to activate a current in the absence of ACh. Because it is assumed that the L247T mutation renders conductive one of the desensitised states, these data indicate that PnIA toxin inhibits the alpha7 by stabilising a desensitised non-conducting state. Activation of the L247T observed with [A10L] further demonstrates that this mutation stabilises another state of the receptor. 1. Hogg et al. (1999) *J. Biol. Chem.*, 274, 36559-36564. 2. Bertrand et al. (1997) *Neuroreport*, 8, 3591-3596.

SEGREGATION OF PHOSPHATIDIC ACID-RICH DOMAINS IN RECONSTITUTED ACETYLCHOLINE RECEPTOR MEMBRANES

J.A. Poveda, J.A. Encinar, A.M. Fernandez, M.L. Molina, R. Mateo, J.M. Gonzalez-Ros

Universidad Miguel Hernandez, Elche, Spain

Purified Acetylcholine Receptor (AChR) from Torpedo has been reconstituted at high (3500:1) and low (560:1) phospholipid to protein molar ratios into vesicles containing egg phosphatidylcholine, cholesterol and different dimyristoyl phospholipids (dimyristoyl phosphatidylcholine, phosphatidylserine, phosphatidylglycerol and phosphatidic acid) as probes to explore the effects of the protein on phospholipid organization by differential scanning calorimetry, infrared and fluorescence spectroscopy. All the experimental results indicate that presence of the AChR protein, even at the higher phospholipid to protein molar ratio, directs lateral phase separation of the monoanionic phosphoryl form of the phosphatidic acid probe, causing the formation of specific phosphatidic acid-rich lipid domains that become segregated from the bulk lipids and whose extent (phosphatidic acid sequestered into the domain, out of the total population in the vesicle) is protein dependent. Furthermore, fluorescence energy transfer using the protein tryptophan residues as energy donors and the fluorescence probes trans-parinaric acid or diphenylhexatriene as acceptors, establishes that the AChR is included in the domain. The other dimyristoyl phospholipid probes, under identical conditions, could not mimic the protein-induced domain formation observed with the phosphatidic acid probe and produce ideal mixing of all lipid components in the reconstituted vesicles. Likewise, in the absence of protein, all the phospholipid probes exhibit ideal mixing behaviour. Since phosphatidic acid along with cholesterol have been implicated in functional modulation of the reconstituted AChR, it is suggested that such a specific modulatory role could be mediated by domain segregation of the relevant lipid classes. Supported by grant PM98-0098 from the DGICYT of Spain.

RAPSYN ESCORTS THE NICOTINIC ACETYLCHOLINE RECEPTOR ALONG THE EXOCYTIC PATHWAY VIA THE ASSOCIATION WITH LIPID RAFTS

S. Marchand¹, A. Devillers-Thiery², S. Pons², J-P. Changeux², J. Cartaud¹

¹Institut Jacques Monod, Universités Paris 6 and 7, ²Institut Pasteur, Paris, France

The 43kDa receptor-associated protein rapsyn is a myristoylated peripheral protein that plays a central role in nicotinic acetylcholine receptor (AChR) clustering at the neuromuscular junction. In a previous work, we demonstrated that rapsyn initially associates with the exocytic pathway and is specifically cotransported with AChR via post-Golgi vesicles targeted to the innervated surface of the Torpedo electrocyte (Marchand et al., 2000). In this work, to further elucidate the mechanisms for sorting and assembly of postsynaptic proteins, we analyzed the dynamics of the intracellular trafficking of fluorescently labeled rapsyn in the transient expressing COS-7 cell system. Our approach was based on fluorescence, time-lapse imaging and immunoelectron microscopies, as well as biochemical analyses. We report that neosynthesized rapsyn associates with the trans-Golgi network compartment and trafficks via vesiculotubular organelles towards the cell surface of COS-7 cells. The targeting of rapsyn organelles appeared to be mediated by a microtubule-dependent transport. Using cotransfection experiments of rapsyn and acetylcholine receptors, we report that these two molecules codistribute within exocytic organelles and are cotargeted to the plasma membrane. Triton extraction on ice and flotation gradient centrifugation demonstrated that rapsyn and AChR are recovered in low density fractions enriched in two raft markers, caveolin 1 and flotillin 1. These data show that sorting and targeting of these two companion molecules are mediated by association with cholesterol-sphingolipid-enriched rafts microdomains. This raises the interesting hypothesis of the participation of the raft machinery in the targeting of signaling molecules at synaptic sites.

IDENTIFICATION AND CHARACTERIZATION OF DIVERSE FAMILY OF NEUROTOXIN-LIKE PEPTIDES FROM THE SOUTH AMERICAN CORAL SNAKE

T. Kubo¹, G. Baptista^{1,2}, X. Yang¹, S. Kobayashi¹, M. Takeda¹, A. Prieto-Da-Silva², T. Yamane²

¹Molecular Neurophysiology Group, AIST Neuroscience Research Institute, Tsukuba, Ibaraki, Japan, ²Center for Applied Toxinology, Butantan Institute, São Paulo, SP, Brazil

Snake venoms are unique mixtures with reference to their biochemical and pharmacological properties. A bite by Elapidae snake often causes serious neurogenic symptoms, such as convulsions and paralysis. The mixture of toxins may target the various synaptic proteins including nicotinic and muscarinic acetylcholine receptors (AChRs), potassium and calcium channels. To elucidate the molecular and physiological basis of the mode of toxin actions, we isolated cDNAs for neurotoxin-like peptides from the venom gland cDNA library of South American coral snake (*Micurus corallinus*). We have identified ten new neurotoxin-like peptides so far, named CTx1 to CTx10. They are 56-67 amino acid residues long in mature form, and having eight cysteine residues in the corresponding position; the cysteine framework is critical for folding into a three-finger structure of neurotoxins. The CTx2 has two additional cysteine residues, which may possibly make the fifth disulfide bridge within the first loop. Seven peptides are more or less similar to the representative neurotoxins: short and long neurotoxins, agAusticepsah-type toxin, muscarin toxin, cardiotoxin. However, the intercysteine interval sequences of the peptides CTx3, CTx4 and CTx8 are fairly diverged from the known neurotoxins. Each recombinant toxin (rCTx) was prepared in *E. coli*, yeast and/or *Xenopus* oocytes and characterized. The peptides interact with acetylcholine binding protein (AChBP) in variable kinetics and blocked ACh-induced muscle- and neuronal-type nicotinic AChR responses expressed in *Xenopus* oocytes.

ORIGIN OF ACETYLCHOLINESTERASE IN THE DEVELOPING NEUROMUSCULAR JUNCTION

M. Jevsek, T. Mars, Z. Grubic

Institute of Pathophysiology, Medical Faculty, University of Ljubljana, Slovenia

Most of the acetylcholinesterase (AChE) in the neuromuscular junction (NMJ) is bound to the basal lamina located between the nerve ending and postsynaptic membrane of the muscle fiber. Potentially, this AChE can originate in both, muscle and/or nerve. Present evidence strongly supports muscular origin of synaptic AChE, however neural origin has never been disapproved and is supported by another line of evidence. In this work we studied the origin of synaptic AChE at the early stages of the NMJ formation. We employed an in vitro model in which motor neurons originating from the embryonic rat spinal cord explants form well differentiated NMJs with human myotubes. By immunocytochemical staining with species specific antibodies against human and rat AChE, we were able to distinguish human AChE (muscle origin) from the rat AChE (neural origin) in the NMJ. Phase-contrast microscopy and fluorescent identification of acetylcholine receptors were used for the visualization of the NMJs. A strong signal was observed after staining with anti-human AChE antibodies indicating relatively strong muscular AChE contribution. Faint signal was observed at the NMJ also after the staining with rat anti-AChE antibodies suggesting neural origin of a part of synaptic AChE. At present we can conclude that at the early stages of NMJ development (3-4 weeks of co-culture) majority of AChE is of muscular origin but that a small part might also be contributed by the motor neuron. Additional experiments will be necessary to assess quantitatively the neuronal origin of AChE in developing NMJ.

MODELS OF THE EXTRACELLULAR DOMAIN OF THE NICOTINIC RECEPTORS AND OF AGONIST AND CA⁺⁺ BINDING SITES

N. Le Novère, T. Grutter, J-P. Changeux

Institut Pasteur, Paris, France

We constructed a three-dimensional model of the amino-terminal extracellular domain of three major types of nicotinic acetylcholine receptor, (alpha7)5, (alpha4)2(beta2)3 and (alpha1)2beta1gamma delta, on the basis of the recent X-ray structure determination of the molluscan Acetylcholine Binding Protein. Comparative analysis of the three models reveals that agonist binding pocket is much more conserved than the overall structure. Differences however exist in the side chains of several residues. In particular, a phenylalanine residue, present in beta2 but not in alpha7, is proposed to contribute to the high affinity for agonists in receptors containing the beta2 subunit. The semi-automatic docking of agonists in the ligand binding pocket of (alpha7)5 led to positions consistent with labeling and mutagenesis experiments. Accordingly, the quaternary ammonium head group of nicotine makes a pi-cation interaction with W148 (alpha7 numbering), while the pyridine ring is close both to the cysteine pair 189-190 and to the complementary component of the binding site. The intrinsic affinities inferred from docking give a rank order epibatidine > nicotine > acetylcholine, in agreement with the experimental values. Finally, our models offer a structural basis for the potentiation by external Ca⁺⁺.

PROBING THE BINDING INTERFACE BETWEEN THE NICOTINIC ACETYLCHOLINE RECEPTOR AND A SHORT ALPHA-NEUROTOXIN THROUGH RECEPTOR-BIOTINYLTAXIN-STREPTAVIDIN TERNARY COMPLEXES

F. Teixeira, A. Menez, P. Kessler

Département d'Ingénierie et d'Etudes des Protéines, CEA/Saclay, Gif-Sur-Yvette, France

We investigated the interacting surface between a short curarimimetic toxin and a muscular-type nicotinic acetylcholine receptor, looking for the ability of various biotinylated *Naja nigricollis* alpha-neurotoxin analogues to bind simultaneously the receptor and streptavidin. All these derivatives, modified at positions 10 (loop I), 27, 30, 33, 35 (loop II), 46, 47 (loop III) or N-terminal still shared high affinity for the receptor, and in the absence of receptor, they all bound soluble streptavidin. However, the proportion of the toxin/receptor complex, that bound onto streptavidin-coated beads, varied both with the location of the modification and with the length of the linker between biotin and the toxin. In the receptor/toxin complex, the concave side of loops II and III was not accessible to streptavidin, contrary to the N-terminus of the toxin and, to a certain extent, to loop I. On the convex face, loop III was the most accessible, while the tip of loop II, especially Arg30 seemed to be closer to the receptor. The present data demonstrate that short toxins neither lay parallel nor strictly perpendicular to the receptor extracellular wall, nor do they penetrate deeply into a crevice. These results fit nicely with 3D-models of interaction between long neurotoxins and their receptors and support the idea that short and long curarimimetic toxins bind in a nearly similar way.

THE AGONIST BIPHASIC DOSE-RESPONSE CURVE OF THE HUMAN ALPHA4BETA2 RECEPTOR BECOMES MONOPHASIC IN THE PRESENCE OF PKC INHIBITORS OR IN LOW LEVELS OF EXTRACELLULAR CALCIUM IONS

I Bermudez, L.M. Houlihan

Oxford Brookes University, Oxford, UK

In the last year it has become widely accepted that the agonist dose-response curve of human alpha4beta2 (ha4b2) nicotinic acetylcholine (nACh) is biphasic. However, the molecular basis for the presence of multiple components in the agonist concentration-response curve, and their pharmacological nature, have not yet been fully determined, although their modulation by phosphorylation and chronic exposure to ligands has received some attention (Mileo, 1995). We have used the potent nicotinic agonist epibatidine to construct a characteristic two-component dose-response curve for ha4b2 nACh receptors expressed in oocytes. The two components were modulated by chronic exposure to both competitive antagonists and agonists, and by Ca^{++} , in a concentration dependent manner. Nicotine upregulated the high affinity component, whereas the high affinity component was abolished by antagonists. To investigate the mechanism whereby chronic ligand exposure modulated the two components, we used activators and inhibitors of Protein Kinase C to modify phosphorylation of the ha4b2 nACh receptor. Inhibition of Protein Kinase C caused a significant reduction in the size of the high affinity component only. This study shows that the two components of the epibatidine dose-response curve for the ha4b2 nACh receptor can be differentially modulated.

NFkB REGULATES THE ACTIVITY OF HUMAN ACETYLCHOLINESTERASE PROMOTER IN MUSCLE

R.C.Y. Choi¹, N.L. Siow¹, A.W.M. Cheng¹, D.C.C. Wan², K.W.K. Tsim¹

¹The Hong Kong University of Science and Technology, Hong Kong, China.

²The Chinese University of Hong Kong, Shatin, Hong Kong, China

Precise expression of biological active acetylcholinesterase (AChE; EC 3.1.1.7) is required for development and maintenance of the neuromuscular junctions. Previous studies reveal that the expression of human AChE in muscle can be regulated by the classical cAMP-dependent pathway that involved cAMP-dependent protein kinase (PKA) and cAMP-responsive element binding protein (CREB), via the regulatory element called cAMP-responsive element (CRE) on promoter of the enzyme. In the present study, we investigated the role of another regulatory element namely NFkB, in regulating the AChE promoter activity. A about 2.2 kb human AChE promoter was tagged with a luciferase reporter gene, and the construct was transfected in chick myotube cultures for analyzing the luciferase activity. The transcriptional activity of human AChE promoter was shown to be regulated by NFkB signaling. One of the possible nerve-derived factors that utilizes this mechanism is the adenosine 5-prime-triphosphate (ATP) and its correspondent receptor called P2Y purinoceptor. All these data suggested that the AChE promoter could be regulated by multiple signaling pathways for precise transcriptional control at the post-synaptic muscle. Acknowledgments: supported by grants from the Research Grants Council of Hong Kong (HKUST 6099/98M, 6112/00M & 2/99C).

MOLECULAR CLONING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNIT GENES FROM THE PEACH-POTATO APHID, MYZUS PERSICAE

M. Kirwan¹, Y. Huang¹, M.S. Williamson¹, A.L. Devonshire¹, J.D. Windass², S. Dunbar², S.J. Lansdell³, N.S. Millar³

¹IACR-Rothamsted, Harpenden, UK, ²Syngenta, Bracknell, UK,

³Department of Pharmacology, University College London, UK

Nicotinic acetylcholine receptors (nAChRs) play a major role in excitatory synaptic transmission in insects and are the primary target site for the recently introduced chloronicotinyl insecticides such as imidacloprid. These compounds displays a high selectivity towards insect nAChRs and are particularly active against 'sucking pests' such as aphids and whiteflies. Despite its commercial importance as a target for insecticides and the recent cloning of nAChR subunit genes from a range of insect species, the insect nAChR is not well characterized at the molecular level and the subunit combinations required to reconstitute functional insect nAChRs are yet to be determined. We are investigating the molecular diversity of nAChR subunit genes in an important agricultural pest, the peach-potato aphid *Myzus persicae*. Five genes have already been cloned as full-length cDNAs (Mpa1-4, Mpb1) and others are currently being isolated based on their homology to nAChR subunit-like genes identified in the full genome sequence of *Drosophila melanogaster*. Heterologous expression studies of the *Myzus* subunit genes in *Drosophila* S2 cells have revealed some evidence for co-assembly of certain alpha/beta subunit combinations, although functional reconstitution of receptor complexes as judged by radioligand binding (epibatidine and imidacloprid) have so far been entirely dependant on co-expression of the *Myzus* alpha subunits with rat beta2 rather than Mpbeta1. This suggests that one or more key *Myzus* nAChR subunits required for functional reconstitution of the native receptor have yet to be cloned, and ongoing efforts to identify and express these subunits will be presented.

NEW ESSENTIAL RESIDUES IN CHOLINESTERASE ACYL POCKET

S.N. Moralev

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Science, Saint-Petersburg, Russia

The statistical analysis of changes of the bimolecular rate constant ($\Delta \lg k_a$) of inhibition of cholinesterases (ChE)—acetylChE from human, mouse and flies *Musca domestica* and *Calliphora vicina*, and horse butyrylChE—by dialkylphosphates (OPI, 67 compounds of 13 series) with the general formula $(AlkO)_2P(O)X$ at elongation of alkyl radicals and change of their branching in comparison with three physical-chemical characteristics (hydrophobicity (hyd), polarity (pol), and volume of the side chain (vol)) of 6 amino acid residues in acyl and alkoxy pockets variable in the studied ChE (No 282, 287, 288, 290, 330, 335 in *Torpedo* ray acetylChE sequence) is performed. It has been shown that depending on structure of alkyl radicals, the rate of ChE interaction with OPI is determined by steric hindrances to sorption (residues 282, 287, 290, 335), hydrophobic interactions (288) or polarity of microenvironment (287). In particular, gradients of the inhibition constants at gradual elongation of alkyls from methyl to hexyl ones are correlated to pol(287), hyd(288), vol(335), vol(282) and vol(287) accordingly. Revealed dependences are statistically significant in most cases; however, rather low values of the correlation coefficient indicate influence of structure of the OPI leaving part. The decrease of the statistical significance with elongation of alkyl radicals seems to be due to an increase of the number of possible conformational states of the OPI molecule. Thus the list of essential residues in ChE acyl pocket has been enlarged.

VARIABILITY OF SUBSTRATE SPECIFICITY IN CHOLINESTERASES OF VERTEBRATES AND INVERTEBRATES

E.V. Rozengart, S.N. Moralev

*Sechenov Institute of Evolutionary Physiology and Biochemistry,
Russian Academy of Sciences, Saint-Petersburg, Russia*

The data on substrate specificity of cholinesterases from vertebrates and invertebrates, obtained during about 40 years work of Soviet school of cholinesterase researchers and from available foreign journals, were summarized. The relative rate of hydrolysis (ratios of V or k_{cat}), relative "affinity" of substrate to active center (ratios of V/K_M or k_i) and relative "affinity" of substrate to peripheral "anionic" site (ratio of K_{SS}) at hydrolysis of the choline (acetyl-, propionyl-, butyrylcholine, acetyl- β -methylcholine) and/or of corresponding thiocholine substrates by 59 cholinesterases from 49 different animals (chordates, insects, mollusks, nematodes) were analyzed. The characteristic features of the enzymes from different groups of animals were revealed. The absence of regular trend of parameters of cholinesterase substrate specificity during evolutionary development was shown. It is supposed, that the evolution of the cholinesterase active center occurs by neutral mutations and results both in divergence, and in convergence of their catalytic properties. This review, named as "Substrate inhibition — one of the aspects of substrate specificity of cholinesterases from vertebrates and invertebrates", is accepted in *J. Evol. Biochem. Physiol.*, 2001, vol.37, no.5 (English version of the Journal is published by Kluwer Academic/Plenum Press).

PERIPHERAL BINDING OF ETHOPROPAZINE TO HORSE SERUM BUTYRYLCHOLINESTERASE

E. Reiner, G. Sinko, A. Stuglin, V. Simeon-Rudolf

Institute for Medical Research and Occupational Health, Zagreb, Croatia

The inhibition of purified horse serum butyrylcholinesterase (BChE) with ethopropazine (0.25-20 μ M) was studied in order to evaluate the binding site(s) on the enzyme. Activities were measured spectrophotometrically with acetylthiocholine (ATCh; 0.05-80 mM) as substrate at 37°C in 0.1 M phosphate buffer pH=7.4. The pS-curve for the ATCh hydrolysis fitted well the Webb equation: K_s and K_{ss} = 0.25 and 2.0 mM respectively, β =3.2. This equation assumes two binding sites for the substrate on the enzyme, catalytic and peripheral, and the β -value above unity indicates apparent substrate activation. Inhibition of BChE with ethopropazine was non-competitive at substrate concentrations up to 1.0 mM. The enzyme-inhibitor dissociation constant was 0.81 μ M. Competition between ATCh and ethopropazine occurred at substrate concentrations above the K_{ss} value for ATCh. Such inhibition pattern indicates binding of ethopropazine to the peripheral, non-productive, site on the enzyme.

2-AMINOPERIMIDINE IS AN EFFECTOR OF CHOLINESTERASES

Y. Shalitin, D. Segal, D. Gur

*Department of Biology, Technion-Israel Institute of Technology, Haifa,
Israel*

Few hydrophobic quaternary nitrogen compounds were reported in the literature as activators of cholinesterases.

We have studied the effect of 2-aminoperimidine (AP), which consists of a guanidine group fused to a naphthalene moiety, on the activity of cholinesterases. AP was found to inhibit the reaction of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with their specific choline esters with K_i in the micromolar range. With a series of p-nitrophenyl (pNP) esters as substrates of BChE the reaction rate was increased in the presence of AP. The maximum rate enhancement, 9.5 fold, was obtained with pNP butyrate in the presence of 10 μ M AP. On the other hand, AP was an efficient inhibitor of the hydrolysis of o-nitrophenyl (oNP) esters. The opposite effect of AP on the hydrolysis of pNP and oNP esters is probably due to a diverse mode of the interaction of the esters with the enzyme's active site in the enzyme-substrate-AP ternary complexes. A spectacular effect of AP was on the covalent inhibition of BChE by diphenyl carbamoyl chloride (DPCC) which inhibits the enzyme with K_i =80,000 1/(M.min). Addition of AP to the inhibition mixture leads to a dramatic enhancement of the inhibition rate up to 300 fold, yielding inhibition rate constant of 2.4×10^7 1/(M.min), which is similar to that obtained by the most powerful inhibitors of cholinesterases.

It seems that AP induces conformational changes in the cholinesterase molecule which can lead to striking activation effects.

SOME CONSIDERATIONS TO MOLECULAR MECHANISM OF CHOLINESTERASE CATALYSIS

N.B. Brovtsyna, E.V. Rozengart, A.A. Suvorov, S.N. Moralev

*Sechenov Institute of Evolutionary Physiology and Biochemistry,
Russian Academy of Science, Saint-Petersburg, Russia*

In due time we have offered the scheme of cholinesterase catalysis supposing formation of octagonal cyclic enzyme-substrate complex by the substrate carboxyl group, serine hydroxyl and histidine imidazole (Brestkin and Rozengart, *Nature*, 1965, V.205, N 4969, P. 388-389). This scheme was conceded by many researchers and cited in several monographies on cholinesterases. However the modern concept of esteratic hydrolysis, based, in particular, on the data of X-ray crystallographic analysis, comprehends participation of catalytic triad Ser-His-Glu and oxyanion hole, which nitrogens forms hydrogen bonds with the substrate carboxylic oxygen. With the purpose to solve finally the problem on possibility of formation of cyclic enzyme-substrate complex, we have carried out researches by the method of molecular mechanics using programs ZMM and MVM (author D.B.Tikhonov, unpublished). It is shown, that the cyclic complex closure is impossible sterically in both investigated variants of interaction of acetylcholine with the triad (with Ser or with Glu). It confirms the scheme, accepted now, of reciprocation of the serine hydroxyl hydrogen during both acylation and deacylations. At the same time it is necessary to note, that the discussed problem on the one or two-proton mechanism of the catalysis is devoid of sense, as the protons in hydrogen bonds, both Ser-His and His-Glu, are delocalized perennially, in free enzyme, in transition state and in acylated enzyme, and it should talk only about shift of the equilibrium. This delocalization of protons in catalytic triad is one of the causes of extremely high rate of the esteratic hydrolysis.

PHOSPHONYLATION OF ACETYLCHOLINESTERASE AND THE PROPENSITY FOR REACTIVATION ANALYZED BY CHIRALITY AND MUTAGENESIS

Z. Kovarik^{1,2}, Z. Radic², H.A. Berman³, P. Taylor²

¹Institute for Medical Research and Occupational Health, Zagreb, Croatia.
²Department of Pharmacology, University of San Diego, CA, USA. ³Department of Biochemical Pharmacology, State University of New York at Buffalo, NY, USA

Mouse acetylcholinesterase (AChE) and its mutants were inhibited with Sp- and Rp- cycloheptyl- (CHMP), isopropyl- (iPrMP), and 3,3-dimethylbutyl- (DMBMP) methylphosphonyl thiocholine enantiomers. Double and triple mutants of AChE had combined substitutions in the acyl pocket (F295L, F297I), choline binding site (Y337A, F338A) and in the active site residue, neighbouring the catalytic serine, F202Q.

The Sp-enantiomers of the methylphosphonate esters are more reactive in forming the conjugate with AChE than Rp-enantiomers. The majority of combined mutants, however, were inhibited by Sp isomers at slower rates than the wild type AChE. Opening of the choline binding site by mutations Y337A/F338A enhanced inhibition rates 2-fold for all inhibitors except for Sp-CHMP which inhibited the mutant at a similar rate as wild type AChE. On the other hand, modification of aromatic residues in the active centre of AChE into aliphatic residues found in butyrylcholinesterase, F295L, F297I and Y337A, enhance inhibition of Rp isomers thus approaching inhibition rates of butyrylcholinesterase. Upon the F297I/Y337A mutations, Rp enantiomers of CHMP and iPrMP became more reactive than Sp enantiomers while reaction with the Sp enantiomers was slightly reduced, displaying inverted stereospecificity. Similar multiple mutations at these positions have been analysed for reactivation by the oximes, 2-PAM and HI-6. Similar to the inactivation rates, the Sp formed conjugates show the more rapid reactivation rates. Certain multiple mutations yield substantial enhancements of reactivation rates. Binary combinations of oximes and these mutant enzymes may form effective scavenging agents.

(Supported by GM18360, DAMD1718014 and a fellowship of the Ministry of Science and Technology of the Republic of Croatia)

THE FIRST TWO NATURALLY OCCURRING ACTIVATORS / REACTIVATORS OF ACETYLCHOLINESTERASE

R. Gupta, S.S. Thakur

Physiology and Medicine Laboratory, Department of Botany,
University of Delhi, India

There is no satisfactory way to provide protection against toxic effects of anticholinesterases (antiChE) in cases of environmental contamination by pesticides, chemical warfare, accidental overdoses of anaesthesia or accidental ingestion of antiChE present in adulterated or contaminated food or fodder. Although some synthetic reactivators of acetylcholinesterase (AChE) are known, no naturally occurring activator or reactivator has been reported thus far. We report isolation; physico-chemical properties and biochemical activities of two novel activator of AChE (EC.3.1.1.7) found in wheat leaves. The compounds have been named as whecheac (wheat ChE activator) and triticheac (Triticum ChE activator). Whecheac is a novel diacylglycerol (DAG). It is an activator of AChE at low concentrations (50 percent activation at 7 nM and 30 nM) but inhibitor at higher concentrations (50 percent inhibition at 10 microM). It is the first report of direct regulation by DAG of any enzyme other than protein kinase C. Whecheac partially reactivates the neostigmine- and succinylcholine- inhibited electric eel AChE (EC. 3.1.1.7). Triticheac is a novel organophosphate (OP). It is the first organophosphate reactivator of AChE in a scenario where organophosphates are almost synonymous with antiChEs. Triticheac has 50 percent activation at 30 microM for electric eel AChE. It also partially reactivates AChE inhibited by neostigmine, succinylcholine and Phosphamidone. Discovery of activation/ reactivation of AChE by naturally occurring chemical compounds has tremendous implications in physiology, therapeutics-- particularly for cholinergic dysfunction, and study of plant-animal co-evolution.

(With inputs from Prof. M.R. Parthasarathi, Department of Chemistry, University of Delhi, Delhi. Supported by a U.G.C. research grant to RG and fellowship from CSIR to SST. Patent applications: 1240/DEL/01 and 1241/DEL/01).

POLYURETHANE IMMOBILIZED ENZYMES: OP SENSING AND DECONTAMINATING MATRIXES

R.K. Gordon¹, B.P. Doctor¹, S.R. Feaster¹, A.T. Gunduz¹, E.D. Clarkson², D.E. Lenz², D.M. Maxwell², T. Cronin³, J.P. Skvorak⁴, M.C. Ross⁴

¹Division of Biochemistry, Walter Reed Army Institute of Research, Silver Spring, MD, USA. ²United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA. ³Chemical Biological Radiological and Nuclear Countermeasures, Technical Support Working Group, Fort Washington, MD, USA. ⁴Medical Chemical Defense Research Program, United States Army Medical Research and Materiel Command, Ft. Detrick, MD, USA

During combat, personnel have been exposed to organophosphates (OPs). Other exposures to chemical toxins include pesticides or terrorist acts in subways or sports events. For successful survival of exposed persons with minimal adverse effects, it is important to have rapid and simple detection of the OPs and also uncomplicated decontamination and detoxification procedures. To accomplish this, we are developing enzyme-immobilized polyurethanes configured as (1) biosensors for OPs or (2) as sponges to soak up and inactivate the OPs. As a biosensor for OPs, the polyurethane matrix is composed of cholinesterase or other OP hydrolyzing enzymes to both indicate the presence of the OP agents, and to differentially indicate the type of OP present in the field. One of the advantages this immobilization technique affords the enzymes is that they are resistant to denaturing events, and are now suitable for sampling OPs in diverse environments such as soil, large bodies of water, as well as conventional airborne contamination. In the second configuration, polyurethane sponges are synthesized with enzymes and agents for external treatment of OP contaminated skin and other sensitive and exposed surfaces. To detoxify OPs, the cholinesterase is combined with oximes so the catalytic activity of OP-inhibited enzyme is continuously restored. Additional post-synthesis components include compounds to improve the extraction of OPs from guinea pig skin. Resulting sponges provided protective ratios of about 15 and 30-fold for soman and VX, respectively, when tested in a guinea pig model. These immobilized enzyme biosensors and sponges, by virtue of their high capacity for enzymes, stability, specificity, sensitivity, and resistance to harsh environmental conditions, can be used under diverse conditions encountered by troops and civilian first responders in the field.

KINETICS OF INTERACTION OF ETHOPROPazine ENANTIOMERS WITH BUTYRYLCHOLINESTERASE AND ACETYLCHOLINESTERASE

G. Sinko¹, Z. Radic², V. Simeon-Rudolf¹, E. Reiner¹, P. Taylor²

¹Institute for Medical Research and Occupational Health, Zagreb, Croatia
²Department of Pharmacology, University of California at San Diego, La Jolla, CA, USA

The association and dissociation rates of (+)ethopropazine and (-) ethopropazine with wild-type mouse and horse butyrylcholinesterases (BChE), and mutant mouse acetylcholinesterase (AChE) were studied in order to analyze elements of stereo selectivity in two similar but distinct enzyme templates. Reaction traces at 23 °C in 0.1 M phosphate buffer pH 7.0 were recorded in millisecond time frame using Applied Photophysics stopped-flow apparatus equipped with fluorescence detection. Dissociation rate constants evaluated for BChEs were three to four-fold faster for (-)-enantiomer (130 (1/min) vs. 30 (1/min) for horse BChE), while association rate constants for enantiomers were similar (1.4 (1/nM*min) vs. 1.0 (1/nM*min) for horse BChE), resulting in lower equilibrium dissociation constant and better binding of (+) ethopropazine with BChE. In the AChE template the preferential binding of (+)ethopropazine was observed with Tyr337Ala mutant, but with an order of magnitude greater stereo specificity. Binding preference for Tyr124Gln AChE mutant, however, was inversed and (-)ethopropazine (Kd of about 1.8 uM) bound several fold better than (+)enantiomer. Inhibition of enzymic acetylthiocholine hydrolysis by ethopropazine enantiomers yielded equilibrium inhibition constants similar to equilibrium dissociation constants derived from stopped-flow rate measurements. In conclusion, active center gorge of AChE, lined with larger number of aromatic residues than the gorge of BChE, provides narrower and more stereo selective environment for binding of ethopropazine.

REACTIVATION STUDY INDICATES THAT THE ORIENTATIONS OF HI-6 MAY DIFFER IN REACTIVATING ACETYLCHOLINESTERASE INHIBITED WITH ORGANOPHOSPHATE AND ORGANOPHOSPHONATES

Chunyuan Luo¹, Ashima Saxena¹, Haim Leader¹, Zoran Radic², Donald M. Maxwell¹, Palmer Taylor², Bhupendra P. Doctor¹

¹Division of Biochemistry, Walter Reed Army Institute of Research, Silver Spring, MD, ²Department of Pharmacology, University of California at San Diego, La Jolla, CA, US Army Medical Research Institutes of Chemical Defense, Aberdeen Proving Ground, MD, USA

The inhibition of acetylcholinesterase (AChE) by organophosphorous (OP) nerve agents (sarin, soman, GF and VX) and pesticides (paraoxon and its analogs) poses a continuous threat due to the possible use in battlefield, terrorist attack or in agriculture. Antidotes containing oxime compounds to reactivate the inhibited enzyme are highly valued for treatment against OP poisoning. One of these reactivators, HI-6, is significantly more effective in alleviating toxicity due to soman and GF compared with the old generation of oximes such as 2-PAM, TMB4 and LüH6. However, HI-6 shows less effective as an antidote for the treatment of OP pesticide poisoning compared to other oximes. The mechanism responsible for this observed selectivity of HI-6 is unknown.

In the present study, the mechanism of HI-6-induced reactivation of AChE-OP conjugates was investigated by using mutant mouse AChEs and different OPs such as paraoxon, 7-(methylethoxyphosphoryl-oxy)-1-methylquinolinium iodide (MEPQ), soman, sarin, and Sp and Rp isopropyl methylphosphonyl thiocholines. The effect of mutations on HI-6-induced reactivation was examined and results indicate that HI-6 can assume two possible orientations in the active-site gorge of AChE to reactivate different types of AChE-OP conjugates. For all enzyme conjugates formed with the Sp enantiomers of organophosphonates, Tyr 124 at the peripheral site is an important element in facilitating reactivation by HI-6 since its mutation to Gln drastically reduced reactivation by HI-6. On the other hand, HI-6 is less effective in reactivating enzyme conjugates formed with the Rp enantiomers and paraoxon, and mutations at the peripheral site do not have a significant effect on reactivation by HI-6. The structure-function correlation suggested by the study with mutant enzymes was further corroborated by reactivation studies with an analog of HI-6, which is devoid of the ether oxygen. These results suggest that the reactivation potency of HI-6 depends on its orientation in the active site gorge, which is dictated by the structure of the OP.

ATTEMPTS TO ENGINEER AN ENZYME-MIMIC OF BUTYRYLCHOLINESTERASE BY SUBSTITUTION OF THE SIX DIVERGENT AROMATIC AMINO ACIDS IN THE ACTIVE CENTER OF ACETYLCHOLINESTERASE

D. Kaplan¹, A. Ordentlich¹, D. Barak², N. Ariel¹, C. Kronman¹, B. Velan¹, A. Shafferman¹

¹Department of Biochemistry and Molecular Genetics, ²Department of Organic Chemistry, Israel Institute for Biological Research, Ness-Ziona, Israel

The active center gorge of human acetylcholinesterase (HuAChE) is lined by 14 aromatic residues, whereas in the closely related human butyrylcholinesterase (HuBChE) 3 of the aromatic active center residues (Phe295, Phe297, Tyr337) as well as 3 of the residues at the gorge entrance (Tyr72, Tyr124, Trp286) are replaced by aliphatic amino acids. For all the prototypical noncovalent active center and peripheral site ligands tested, the hexa-mutant of HuAChE (Y72N/Y124Q/W286A/F295L/F297V/Y337A) displayed a reactivity phenotype closely resembling that of HuBChE. These results support the accepted view that the active center architectures of AChE and BChE differ mainly by the presence of a larger void space in BChE. Nevertheless, reactivity of the hexa-mutant HuAChE toward the substrates acetylthiocholine and butyrylthiocholine and the transition state analog m-(N,N,N-trimethylammonio)trifluoro-acetophenone (TMTFA), is about 45-170-fold lower than that of HuBChE. Most of this reduction in reactivity is manifested by the triple active center mutant F295L/F297V/Y337A. We propose that the hexa-mutant HuAChE, unlike BChE, is impaired in its capacity to accommodate certain tetrahedral species in the active center. This impairment is probably related to the enhanced mobility of the catalytic histidine His447, as observed in molecular dynamics simulations of the hexa-mutant or the F295L/F297V/Y337A HuAChE enzyme but not in the wild type HuAChE.

This work was supported by the U.S. Army Research and Development Command, Contract DAMD17-96-C-6088 and DAMD17-00-C-0021 (to A.S.)

KINETIC AND X-RAY CRYSTALLOGRAPHY STUDIES ON THE INTERACTION OF CHOLINESTERASES WITH THE ANTI-ALZHEIMER DRUG RIVASTIGMINE

P. Bar-On¹, M. Harel², C.B. Millard^{1,3}, A. Enz⁴, J.L. Sussman², I. Silman¹

Depts. of ¹Neurobiology and ²Structural Biology, Weizmann Institute of Science, Rehovot, Israel, ³US Army Medical Research Institute of Infectious Diseases (USAMRIID), USA, ⁴Novartis Pharma, Basel, Switzerland

[*S*]-N-ethyl-3-[(1-dimethyl-amino)ethyl]-N-methylphenyl-carbamate hydrogen tartrate, (SDZ ENA-713, Exelon[®], rivastigmine) a carbamate inhibitor of acetylcholinesterase (AChE), is already used for Alzheimer's disease treatment in Europe and in the USA. Kinetic and structural characterization of the interaction of rivastigmine with cholinesterases has been performed. Rivastigmine slowly carbamylates *Torpedo californica* (Tc) AChE ($k_i = 2.0 \text{ M}^{-1}\text{min}^{-1}$), whereas the bimolecular rate constant for human AChE is more than 1600-fold higher ($k_i = 3,300 \text{ M}^{-1}\text{min}^{-1}$), and for human butyrylcholinesterase and for *Drosophila melanogaster* AChE even greater ($k_i = 9 \cdot 10^4$ and $5 \cdot 10^5 \text{ M}^{-1}\text{min}^{-1}$ respectively). Reactivation of all four enzymes inhibited by rivastigmine is unusually extremely slow, e.g. only 4% in 48 h for TcAChE. The crystal structure of TcAChE and rivastigmine was solved to a 2.2 Å resolution. It revealed a binary complex in which the carbamyl moiety is covalently bound to the active site serine, and the leaving group, [*S*]-3-[(1-dimethylamino)ethyl]phenol (NAP), is retained within the 'anionic' site. Moreover, an unexpected and significant movement of the active site histidine (H440) away from its normal H-bonded partner (E327) was observed. The distance from H440Nδ to E327Oε in the X-ray structure increased relative to the native TcAChE, from 2.5 to 4.0 Å. The crystallographic observation provides a structural explanation for the slow rate of reactivation, which may also be valid for the three other cholinesterases studied.

MALDI-TOF/MS ANALYSIS OF ACETYLCHOLINESTERASE-LIGAND CONJUGATES: A TOOL FOR RESOLUTION OF MECHANISTIC PATHWAYS

E. Elhanani¹, A. Ordentlich¹, O. Dgany¹, D. Kaplan¹, Y. Segal², R. Barak³, B. Velan¹, A. Shafferman¹

¹Department of Biochemistry and Molecular Genetics, ²Department of Organic Chemistry, ³Department of Analytical Chemistry, Israel Institute for Biological Research, Ness-Ziona, Israel

Understanding reaction pathways of phosphorylation and reactivation of AChE and "aging" of the corresponding AChE adducts is both a biochemical and a pharmacological challenge. Here we describe experiments which allowed to resolve some of the less well understood reaction pathways of phosphorylation, and "aging" of acetylcholinesterase (AChE) involving phosphoramidates (P-N agents) such as tabun or the widely used pesticide methamidophos. Tryptic digests of phosphorylated AChEs (from human and *Torpedo californica*), ZipTip peptide fractionation and Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF/MS) enabled reproducible signal enrichment of the isotopically resolved peaks of organophosphoramidate-conjugates of the AChE active site Ser peptides. For tabun and its hexadeuterio-analog we find, as expected, that the two phosphoramidate adducts of the active site peptide differ by 6.05 mass units but following aging we find that the two corresponding phospho-peptides have identical molecular weights. We further show that the aging product of paraoxon-AChE adduct is identical to the aging product of the tabun-AChE conjugate. These results unequivocally demonstrate that the pathway of aging of tabun adducts of the human or the *Torpedo californica* AChEs proceeds through P-N bond scission. For methamidophos we show that phosphorylation of AChE involves elimination of the thiomethyl moiety and that the spontaneous reactivation of the resulting organophosphate adduct generates the phosphorous free AChE active site Ser-peptide.

This work was supported in part by the U.S. Army Research and Development Command, Contract DAMD17-00-C-0021 (to A.S.).

INFLUENCE OF WATER ON THE FUNCTION OF ACETYLCHOLINESTERASE

R.H. Henchman¹, K. Tai¹, T. Shen^{1,2}, J.A. McCammon¹

¹Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, and Department of Pharmacology, University of California, San Diego, La Jolla, CA, USA, ²Department of Physics, University of California, San Diego, La Jolla, CA, USA

A 10 ns trajectory from a molecular dynamics simulation is used to examine the structure and dynamics of water around acetylcholinesterase to determine what influence water may have on its function. Particular emphasis is placed on water in the active site gorge to understand how water may affect ligand entry and binding. Despite the confining nature of the deep active site gorge, ligand entry appears to be aided by a number of water properties - fluctuations in the population of gorge waters, moderate mobility of water in the entrance to the gorge, reduced water hydrogen bonding ability, and transient cavities in the gorge. While a ligand is significantly slowed down by the less mobile water, this effect does not appear to be large enough to be rate limiting for enzyme catalysis of acetylcholine. Images and further information are available at the website <http://mccammon.ucsd.edu/>.

X-RAY STRUCTURE OF TORPEDO AChE COMPLEXED WITH BIFUNCTIONAL LIGANDS RELATED TO HUPA: NOVEL DRUGS FOR TREATMENT OF ALZHEIMER'S DISEASE

D.M. Wong¹, H.M. Greenblatt¹, D. Shaya¹, P.R. Carlier², Y.-P. Pang³, Y.-F. Han⁴, I. Silman¹, J.L. Sussman¹

¹Depts of Structural Biology and Neurobiology, Weizmann Institute of Science, Rehovot, Israel, ²Dept of Chemistry, Virginia Tech, Blacksburg, VA, USA, ³Dept of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA, ⁴Dept of Biochemistry, Hong Kong University of Science and Technology, Kowloon, Hong Kong

Reversible AChE inhibitors of AChE slow the cognitive decline experienced by Alzheimer patients. (-)-Huperzine A (HupA), an alkaloid isolated from the clubmoss *Huperzia serrata*, is one such inhibitor, but the search for more potent and selective drugs continues.

Based on the bivalent strategy and on structural studies on TcAChE, dimerization of a pharmacologically inactive fragment of HupA (monomer E) produced a drug with twice the potency of the natural product. We soaked two alkylene-linked bis-HupA-like AChE inhibitors, (S,S)-(-)-E12E and (S,S)-(-)-E10E, into trigonal TcAChE crystals and solved the X-ray structures using the difference Fourier technique, both to 2.15 angstroms resolution. The structures revealed one E unit bound to the 'anionic' subsite of the active site, at the bottom of the active-site gorge, adjacent to Trp84, as seen for the HupA-TcAChE complex, and the second E unit adjacent to Trp279 in the 'peripheral' anionic site at the top of the gorge, with both bifunctional molecules thus spanning the active-site gorge. The results confirm that the increased affinity of the dimeric HupA analogs for AChE is conferred by binding to the two 'anionic' sites of the enzyme.

THE FLUCTUATING SYNAPSE

K. Kaufmann

Abteilung 081, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany

The observation of single molecules invites to replace molecular model mechanisms. Molecular dynamics look very similar, from the view-point of spectroscopy, as membranes look from the view-point of electrodes (Rigler 1997). Single-channel-like traces appear in DNA, enzymes, single catalytic reaction steps, membrane proteins, alike in patch-clamped membranes and lipid bilayers (Nobel conference 1999). Is there one ubiquitous law? I show it is the Boltzmann-Einstein-law of the proper molecular interfaces. Aqueous hydration layers e.g. will then hydrolyse specific "substrates" by the same law that causes channel-like recordings observed by electrodes when the "reactive adsorbants" induce a strongly fluctuating "transition" state of the interface. From the view-point of electrodes (Kaufmann 1988), entropy explains receptor function, forces explain signal propagation, and the fluctuations explain channel-like recordings. The physical mechanism of cholinergic transmission (Kaufmann and Silman 1980) is generalized to the physical entropy law of catalytic fluctuations induced by acetylcholine. The induction of channel-like fluctuations by acetylcholinesterase activity follows. The origin of cholinergic excitations was hitherto pictured into molecular model mechanisms. However, alike in Einstein's law of Brownian motion, single molecules function after the law of proper entropy that unifies catalysis and membrane excitation.

CRYSTALLIZATION AND DETERMINATION OF THE X-RAY STRUCTURE OF HUMAN AChE

H. Dvir¹, G. Kryger¹, J.L. Johnson², T.L. Rosenberry², I. Silman¹, J.L. Sussman¹

¹Department of Structural Biology and Neurobiology, Weizmann Institute of Science, Rehovot, Israel, ²Department of Pharmacology, Mayo Clinic, Jacksonville, FL, USA

For certain AChE inhibitors, crystallographic studies of their complexes with AChE from a particular species may fail to explain differences in binding affinity observed for AChEs from a different species. Thus, it would be preferable to study the binding affinity in solution and the X-ray structure of the complex for AChE from the same species. To date, the only structural reports on complexes of AChE with potential drugs for the management of Alzheimer's disease (AD) are of AChE from *Torpedo californica* (Tc), whereas studies on complexes with human AChE (hAChE) would obviously be preferable. We have recently crystallized a recombinant catalytic domain (556 residues) of hAChE, and solved its X-ray structure at 3.2 Å resolution by molecular replacement. The data revealed a P6(1) space group with unit cell dimensions: 210.9 210.9 115.3 angstrom and angles of 90, 90, 120 degrees. The asymmetric unit contains a dimer with a non crystallographic 2-fold symmetry similar to the crystallographic 2-fold symmetry previously seen for the native dimer. X-ray data have been collected from native hAChE crystals soaked with the anti-Alzheimer drugs: (-)-huperzine A, tacrine, huprine X and ENA-713. The structure determination of these complexes is currently being pursued.

3D STRUCTURE OF TORPEDO CALIFORNICA ACETYLCHOLINESTERASE COMPLEXED WITH HUPRINE X

H. Dvir¹, D.M. Wong¹, M. Harel¹, X. Barri², M. Orozco³, F.J. Luque², P. Camps⁴, T.L. Rosenberry⁵, I. Silman⁶, J.L. Sussman¹

¹Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel, ²Fisicoquímica, Barcelona University, Barcelona, Spain, ³Bioquímica, Barcelona University, Barcelona, Spain, ⁴Química Farmacéutica, Barcelona University, Barcelona, Spain, ⁵Department of Pharmacology, Mayo Clinic, Jacksonville, FL, USA, ⁶Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Huprine X is a novel AChE inhibitor, with one of the highest affinities reported for a reversible inhibitor (Camps et al. Mol. [2000] Pharmacol. 57, 409-417). It is a synthetic hybrid containing the 4-aminoquinoline substructure of one anti-Alzheimer drug, tacrine, and a carbobicyclic moiety resembling that of another, (-)-huperzine A. The crystal structure of its complex with Torpedo californica AChE (TcAChE), determined to 2.1 angstrom, shows that huprine X binds to the anionic site, and also hinders access to the esteratic site. Its aromatic portion occupies the same binding site as tacrine, whereas the carbobicyclic unit occupies the same binding pocket as (-)-huperzine A. Inhibition data show that huprine X binds human AChE (hAChE) and Torpedo AChE 28-fold and 54-fold, respectively, more tightly than tacrine. This difference stems from the fact that the aminoquinoline moiety of huprine X makes interactions similar to those made by tacrine, but additional bonds to the enzyme are made by the huperzine-like substructure and the chlorine atom of huprine X. Furthermore, both tacrine and huprine X bind more tightly to TcAChE than to hAChE, suggesting that their quinoline substructures interact better with Phe330 in TcAChE than with Tyr337 in hAChE.

bis-ACTING GALANTHAMINE DERIVATIVES AS IMPROVED DRUGS IN THE SYMPTOMATIC TREATMENT OF ALZHEIMER'S DISEASE

H.M. Greenblatt¹, C. Guillou³, B. Badet³, C. Thal³, I. Silman², J. L. Sussman¹

¹Department of Structural Biology, ²Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel, ³Institut de Chimie des Substances Naturelles, C.N.R.S, Gif-sur-Yvette, France

The alkaloid galanthamine (GAL), isolated from the *Amaryllidaceae* family of plants, shows strong, reversible anticholinesterase activity. As such it has been tested as a possible alternative to current anticholinesterases such as Aricept¹, used in the palliative treatment of Alzheimer's Disease. GAL is already in use in Austria and has been approved for use in the UK and the USA, under the trade name Reminyl¹. It interacts with several residues in the active site of acetylcholinesterase (AChE) at the bottom of the "gorge", including Trp84, which binds the quaternary ammonium group of acetylcholine. In an effort to improve the efficacy of this drug, derivatives have been synthesized with the aim of interacting with both the active site and the second cation-binding site at the top of the gorge of AChE, viz., the peripheral binding site. The crystal structures of complexes of two such compounds with *Torpedo californica* AChE have been solved and refined and will be presented.

LIGAND INDUCED CONFORMATIONAL CHANGES IN THE OMEGA LOOP OF ACETYLCHOLINESTERASE REVEALED BY FLUORESCENCE SPECTROSCOPY

J. Shi¹, Z. Radic¹, A. Boyd¹, D.A. Johnson², P. Taylor¹

¹Dept. of Pharmacology, UC San Diego, La Jolla, CA, USA

²Dept. of Biomedical Science, UC Riverside, Riverside, CA, USA

We have used a combination of cysteine substitution mutagenesis and site-specific fluorescence labeling to characterize the structural dynamics of mouse AChE. Cysteines were substituted at positions 262 (distal disulfide loop), 124 and 287 (gorge entry and rim), 76, 81 and 84 (outer rim of the omega loop). In turn, these Cys residues have been specifically labeled with fluorophores, and fluorescence spectra and decay of anisotropy of the fluorescent side-chains analyzed in relation to the kinetic parameters of ligand binding. Residue 262 shows rapid decay of anisotropy and no evidence for a global conformational change upon ligand binding. Ligands which occlude the gorge opening, such as fasciculin or extended bisquaternary ligands, cause blue shifts in the spectra of acrylodan when conjugated to residues 124 and 287; this reflects an increase in local solvent exclusion at these positions by the bound ligand. Unexpectedly, the binding of active center ligands and fasciculin induce a large red shift in emission of acrylodan conjugated at the three outer residues on the omega loop, 76, 81 and 84, indicating an enhanced solvent exposure upon ligand binding. Concomitantly, time resolved fluorescence anisotropy reveals a more rapid rate of anisotropy decay at positions 81 and 84 upon active site ligand binding. Thus, ligand binding at the active center induces a local change in conformation of the omega loop not revealed in crystal structures.

COMPARISON OF TWO REACTION SCHEMES FOR THE HYDROLYSIS OF ACETYLTHIOCHOLINE BY BUTYRYLCHOLINESTERASE

V. Simeon-Rudolf¹, G. Sinko¹, A. Stuglin¹, J. Stojan², M. Golicnik², E. Reiner¹

¹Institute for Medical Research and Occupational Health, Zagreb, Croatia.

²Medical Faculty, University of Ljubljana, Slovenia

Catalytic parameters calculated from the schemes derived by Webb (Model A) and by Stojan (Model B) were compared for the hydrolysis of acetylthiocholine (ATCh) by purified horse serum butyrylcholinesterase (BChE) (measured by conventional and stopped-flow techniques). In Model A the acetylated enzyme is omitted from the scheme, while in Model B the Michaelis complex is omitted. The enzyme-substrate dissociation constants K_s and K_{ss} in Model A were 0.25 and 2.0 mM (conv. tech.) and 0.17 and 6.3 mM (stopped-flow tech.). These were attributed to binding of ATCh to the catalytic and peripheral site of BChE. The constants K_1 and K_2 in Model B (stopped-flow tech.) were 0.223 and >1000 mM. These are attributed to binding of ATCh to an unidentified site in the free enzyme and to the peripheral site in the acetylated enzyme. As the K_s and K_1 values are almost the same, both constants are likely to refer to the same enzyme-substrate complex. The constants K_{ss} and K_2 are very different; they are both attributed to peripheral binding of ATCh, but they obviously refer to different complexes. Both models postulate that peripheral binding of a substrate affects rate constants of substrate hydrolysis; this also follows from the evaluated constants for ATCh hydrolysis.

QUANTAL ACETYLCHOLINE RELEASE THROUGH MEDIATOPHORE PROTEOLIPID OVER-EXPRESSED IN NEUROBLASTIC CELLS

A. Bloc, J. Falk-Vairant, M. Malo, M. Israel, Y. Dunant

¹Departement de Pharmacologie, CMU, Geneve, Switzerland.

²Laboratoire de Neurobiologie Cellulaire and Moléculaire, CNRS, Gif-Sur-Yvette, France

This review summarizes a series of experiments demonstrating that reconstitution of mediatophore in transfected cells support quantal acetylcholine (ACh) release. Mediatophore is a proteolipid initially identified in presynaptic membrane of Torpedo cholinergic nerve terminals. It is formed by homo-oligomeric assembly of 15-16 kDa subunits showing a very high homology with either the 'c' subunit of the V-ATPase transmembrane sector (Vo) and the subunit of a recently identified proteolipid fusion pore in yeast vacuoles. Transfection of the Torpedo 15-16 kDa subunit in non cholinergic neuroblastoma cells (N18TG-2) enable them to perform rapid, Ca²⁺-dependent and quantal release of ACh from a cytosolic pool, in response to electrical stimulation. Transfection of choline acetyltransferase (ChAT) supports ACh release only if mediatophore is transfected as well. Furthermore, evoked release by mediatophore transfected cells is modulated by co-expression of ChAT but also vesicular ACh transporter (VACHT). Together these data indicate that mediatophore proteolipid plays a central role in the process of quantal ACh release. Mediatophore may either work as an transmitter permeable channel using cytosolic transmitter, or form a fusion pore which can allow 'kiss and run' or full exocytosis of synaptic vesicles. One question remains : can these different modes of release co-exist at a same active zone, depending on the presynaptic activity and vesicle life cycle, or are they differentially expressed in specific types of synapses according to their architecture and function?

Supported by the FNRS Grant #31 57135 99

CAPILLARY ZONE ELECTROPHORESIS DETECTS UNWANTED CHOLINESTERASE-BOUND HIDDEN LIGANDS THAT MODULATE ENZYME CONFORMATIONAL STABILITY

D. Rochu¹, F. Renault¹, C. Bon², P. Masson¹

¹CRSSA, Unite d'Enzymologie, La Tronche, ²Institut Pasteur, Unite des Venins, Paris, France

Proteins of pharmacological interest have to exhibit their nakedness to become therapeutic drugs. Thus, detection of compounds present in biopharmaceuticals is of central concern. Among them, cholinesterases (ChEs) are enzymes of major importance for detoxification of poisonous esters. ChEs are characterized by an asymmetrical distribution of charged residues, thought to increase enzyme efficiency, and a remarkable conformational plasticity, to allow adjustment of the active site and the allosteric control of activity. Likewise, ChEs display high catalytic efficiency of an active site positioned at the bottom of a deep gorge. The gorge can be partially or fully occupied by ligands (substrates or inhibitors). Accordingly, a suitable method allowing to detect unwanted ligands and their influence on the functional conformation and stability of these enzymes was essential. We have developed a capillary zone electrophoresis (CZE) approach for that purpose [1]. The factors causing discrepancies between data for thermal unfolding of ChE by electrophoretic and by calorimetric methods were investigated. The presence of unwanted hidden ligands bound to purified cholinesterases was first demonstrated. The role of hidden bound ligand in stabilization of AChE samples was emphasized. Our results raised several questions concerning the real conformation of the native state of enzymes such as ChEs. Finally, CZE was proven to be a pertinent tool to validate the conformity of purified enzymes to a status of biopharmaceutical. [1] Rochu D. et al. 2002, Electrophoresis, in press. This work was supported in part by DSP/STTC CO No 99-CO029 to P.M.

STIMULATION OF NICOTINIC RECEPTORS INDIRECTLY INCREASES ACETYLCHOLINE RELEASE IN RAT STRIATUM

V. Dolezal, V. Zemlickova, S. Tucek

Institute of Physiology, CAS, Prague, Czech Republic

We investigated how the release of prelabelled acetylcholine (ACh) from superfused striatal slices is affected by nicotine. ACh release was evoked by mild depolarization (26.5 mM KCl for 1 min). The effect of KCl was partly blocked by tetrodotoxin (TTX), suggesting that it was due both to direct depolarization of nerve terminals and to enhanced impulse activity in cholinergic neurons. Low concentration of nicotine (0.01 mM) acting during potassium depolarization diminished ACh release and this effect was prevented by D2 dopamine receptor antagonist domperidone. In contrast, high concentration of nicotine (0.1 mM) facilitated ACh release even in the absence of domperidone. This effect of nicotine was prevented by TTX suggesting that it is indirect, mediated by impulse propagation evoked by the action of another transmitter. Pulses of glutamate (1 min) induced concentration-dependent ACh release which was not influenced by nicotine. Glutamate-evoked ACh release was substantially higher in a medium containing 0.001 mM glycine and no magnesium. Under these conditions, favouring the activation of NMDA glutamate receptors, nicotine effect was present during mild potassium depolarizations but not under resting conditions or at full depolarization. Our data are best interpreted on the assumption that nicotine increased ACh release by stimulation of nicotinic receptors on glutamatergic nerve terminals, leading to enhanced release of glutamate and increased impulse activity in cholinergic neurons. Nicotinic receptors on striatal glutamatergic nerve terminals perhaps play a role in the mechanism of striatal cholinergic hyperactivity in Parkinson's disease. Supported by grants GACR 305/01/0283 and GAAV 5011206

CRYSTAL STRUCTURE OF TORPEDO CALIFORNICA ACETYLCHOLINESTERASE WITH A NOVEL GALANTHAMINE DERIVATIVE: IMPLICATIONS FOR THE DESIGN OF NEW ANTI-ALZHEIMER DRUGS

M.C. Siotto¹, C. Bartolucci¹, D. Lamba^{1,2}

¹Istituto di Strutturistica Chimica 'G. Giacomello', C.N.R., Roma,

²International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Galanthamine, a tertiary alkaloid extracted from several species of has received recent attention as a centrally acting, selective, competitive, and reversible showing to produce significant improvement of cognitive performances in Alzheimer's disease patients. This compound is less potent than tacrine and E2020 but has excellent pharmacological and pharmacokinetic profiles and exhibits very low hepatotoxicity and fewer side effects. Galanthamine, together with physostigmine, is one of very few drugs showing a dual activity, acting not only as an inhibitor of AChE but also as an allosteric potentiator of the nicotinic response induced by ACh and competitive agonists. The crystal structure of Torpedo californica AChE with galanthamine has been determined [1,2] and computerized molecular docking studies successfully predicted its bound conformation [3]. Structure-based drug design is an important tool in the development of second generation candidate drugs based on a lead compound. The crystal structure of Torpedo californica AChE with a novel galanthamine derivative was solved to 2.3 Angstroms resolution. The structure revealed that the galanthamine moiety binds at the base of the active site gorge, interacting with both the acyl-binding pocket and the choline binding site. The charged nitrogen of the piperidine moiety, tethered to galanthamine via a functional linker, makes a cation- π interaction with the phenyl ring of Tyr334. [1] H. M. Greenblatt et al. (1999) FEBS Lett. 463, 321-326. [2] C. Bartolucci et al. (2001) Proteins 42, 182-191. [3] C. Pilger et al. (2001) J. Mol. Graph. and Modell. 19, 288-296. This work was supported by Sanochemia Pharmazeutika AG, Vienna, Austria.

X-RAY STRUCTURE OF SOMAN-AGED HUMAN BUTYRYLCHOLINESTERASE

F. Nachon¹, Y. Nicolet², P. Masson¹, J.-C. Fontecilla-Camps², O. Lockridge³

¹Centre de Recherche du Service de Santé des Armées, La Tronche, France,

²Institut de Biologie Structurale, Grenoble, France, ³Eppley Institute UNMC, Omaha, NE, USA

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) phosphorylated by branched organophosphates (OP) undergo a dealkylation reaction ('aging'), leading to OP-enzyme conjugates that cannot be reactivated by oximes. Previous studies from our laboratory revealed that aged-BChE conjugates are more stable to high temperature and pressure than the native enzyme. The increase in stability was shown to be related to changes in the water structure network in the active site gorge and to the formation of a salt bridge between PO⁻ and protonated His438. Such a bridge was observed in the crystal structure of soman-, sarin- and DFP-aged AChE. These x-ray structures also revealed that the reactivation of aged conjugates was prevented by the stabilizing effect of H-bonding between a P bound oxygen and the oxyanion hole, and by the 'drying' effect of the acyl-binding pocket, preventing the nucleophilic attack. To complete our understanding of the molecular mechanism of the aging reaction of phosphorylated BChE, the X-ray structure of an engineered form of human BChE has been determined. The structure of the soman-BChE aged conjugates was solved to 2.4 Å resolution and compared to the aged conjugates of Torpedo californica AChE. Because the aged conjugate has no activity, we used this feature to determine the binding location of butyrylthiocholine in the active site of the phosphorylated enzyme. The structure of the ternary complex aged BChE/butyrylthiocholine was solved to 2.3 Å resolution. This later structure was aimed to provide new information on the peripheral site of BChE and the related phenomenon of substrate activation.

TETANIC FADE IS REVEALED BY BLOCKING PRESYNAPTIC NICOTINIC RECEPTORS CONTAINING ALFA4BETA2 AND ALFA3BETA2 SUBUNITS AFTER REDUCING THE SAFETY FACTOR OF NEUROMUSCULAR TRANSMISSION

M. Faria, L. Oliveira, M.A. Timoteo, M.G.B. Lobo, P. Correia-de-Sa

Laboratório de Farmacologia, UMIb, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal

The effects of subtype specific nicotinic receptor (nAChR) antagonists were studied on nerve-evoked tetanic (50Hz, 5sec) contractions and [3H] acetylcholine release from rat isolated hemidiaphragms. nAChR antagonists reduced tetanic peak tension with a rank potency order of alfa-bungarotoxin(BTX)>d-tubocurarine(TC) >> mecamylamine(Meca) > hexamethonium(Hex). Depression of tetanic peak tension by dihydro-beta-erythroidine(DHbE, 0.03-10 microM, an alfa4beta2 and alfa3beta2 antagonist), methyllycaconitine(MLA, 0.003-3 microM, an alfa7 antagonist) and alfa-conotoxin MII(CTX MII, 1-300 nM, a selective alfa3beta2 antagonist), did not exceed 30%. TC (0.1-0.7 microM), Meca (0.1-300 microM) and Hex (0.03-3 mM) induced both tetanic fade and peak tension depression. With DHbE (0.03-10 microM) and CTX MII (1-300 nM), tetanic fade was only evident after decreasing the safety factor of neuromuscular transmission (MgCl2, 6-7 mM). Neither BTX (3-100 nM) nor MLA (0.003-3 microM) produced tetanic fading. The antagonist rank potency order to reduce (50%) evoked [3H]-ACh release from motor nerve terminals, was CTX MII(100 nM)>TC(1 microM), DHbE(1 microM)>Hex(1 mM); BTX (300 nM) failed to affect ACh release. The results suggest that neuromuscular block is linked to the activation of BTX-sensitive nAChRs containing alfa1-subunits, whereas preferential blockade of neuronal alfa4beta2- and alfa3beta2-containing receptors cause tetanic fade by reducing nicotinic autofacilitation.

Work supported by FCT.

STUDIES ON DYNAMICAL TRANSITIONS IN CHOLINESTERASES

F. Gabel¹, M. Weik¹, L. Brochier¹, D. Fournier², P. Masson³, B. P. Doctor⁴, I. Silman⁵, G. Zaccai¹

¹Institut de Biologie Structurale, Grenoble, France, ²Groupe de Biochimie des Protéines, Université Paul Sabatier, Toulouse, France, ³Unité d'Enzymologie, CRSSA, La Tronche, France, ⁴Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC, USA, ⁵Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel

Acetylcholinesterase (AChE) hydrolyses the neurotransmitter, acetylcholine, very rapidly, as required for termination of impulse transmission at cholinergic synapses. Its 3D structure reveals a deeply buried active site accessed by a narrow gorge [1]. This unanticipated structural characteristic raises cogent questions concerning traffic of substrates and products to and from the active site. It is obvious that substantial 'breathing' motions of the protein are required for penetration of substrates to the active site via the gorge and for clearance of products via routes as yet undetermined. It is, therefore, important to characterise the dynamics of the enzyme on various time scales. We have chosen incoherent elastic neutron scattering (IENS) [2] to investigate global atomic dynamics of both hydrated *Drosophila melanogaster* AChE (DmAChE) and of DmAChE dried by lyophilization as a function of temperature, and compared them to corresponding samples of human butyrylcholinesterase, an enzyme structurally very similar to AChE whose biological function is still unknown. The atomic mean square displacements (MSD) obtained by this technique reveal a dynamical transition for both hydrated samples. Such a transition has been observed previously for myoglobin [3], as well as for bacteriorhodopsin [4], and has been associated with the onset of biological function. Surprisingly, MSDs of the dry samples exceed those of their hydrated counterparts in the intermediate temperature range. Future objectives include theoretical interpretation of these findings, as well as investigation by IENS of the influence of covalently and reversibly bound inhibitors on the dynamics of these enzymes. [1] Sussman J.L., Harel M., Frolow F., Oefner C., Goldman A., Toker L. & Silman I. (1991). Atomic structure of acetylcholinesterase from *Torpedo californica*: A prototypic acetylcholine-binding protein. *Science* 253, 872-879. [2] Zaccai, G. (2001). How Soft is a Protein? A Protein Dynamics Force Constant Measured by Neutron Scattering. *Science* 288, 1604-1607. [3] Doster W., Cusack S. & Petry W. (1989). Dynamical transition of myoglobin revealed by inelastic neutron scattering. *Nature* 337, 754-756. [4] Ferrand M., Dianoux A.J., Petry W. & Zaccai G. (1993). Thermal motions and function of bacteriorhodopsin in purple membranes: Effects of temperature and hydration studied by neutron scattering. *Proc. Natl. Acad. Sci. USA* 90, 9668-9672.

MOLECULAR CHARACTERISATION OF ACETYLCHOLINESTERASE FROM THE PEACH-POTATO APHID MYZUS PERSICAE(SULZ.)

M.C. Andrews, C.G. Bass, M.S. Williamson, G.D. Moores

IACR-Rothamsted, Harpenden, Herts, UK

The peach-potato aphid, *Myzus persicae*, is an extremely important pest that occurs worldwide causing direct feeding damage to many field and glasshouse crops. It is, more importantly, a major vector of many plant viruses including virus yellows in sugarbeet. A modified acetylcholinesterase (AChE) that confers specific insensitivity to pirimicarb and triazamate has been previously reported in *Myzus persicae*. In order to determine the genetic basis of this insensitivity, the AChE gene was amplified from both sensitive and insensitive forms using RT-PCR. However, no mutations were identified which could account for this insensitivity. The presence of a second gene was thus proposed. Alternative degenerate primers were designed based on published ace sequences from both vertebrate and invertebrate organisms. This led to the amplification of a partial sequence that differed from *Myzus persicae* sequence. Gene specific primers have been designed based on this sequence to characterise any mutations found in an insensitive form of the enzyme. Additionally, AChE has also been purified using affinity chromatography. The results of direct sequencing of this AChE protein is compared with amplified sequence from PCR to verify the second ace sequence.

PECULIARITIES OF KINETIC BEHAVIOUR OF FISH'S/ABRAMIS BALLERUS/ BLOOD SERUM CHOLINESTERASE

V.D. Tonkopi

*Institute of Limnology, Russian Academy of Sciences,
St. Petersburg, Russia*

The cholinesterase (ChE) of fish's blood serum is the typical acetylcholinesterase (AChE) with the same substrate specificity as AChE from human erythrocytes. On the other hand, the blood serum of some fish's blood plasma contains mainly butyrylcholinesterase (BuChE). The blood plasma ChE (FChE) of fishes (blue bream - *Abramis ballerus*, roach - *Rutilus rutilus*) was purified for study of kinetic behaviour and sensitivity to antiChE compounds. The kinetics of choline and thiocholine ethers hydrolysis has been studied at the presence of new purified (FChE). The sensitivity of enzyme to organophosphates, carbamates and reversible ChE inhibitors has been determined. The results of this and the following experiments indicate that the new purified FChE can be classified as BuChE, so the velocity of butyrylcholine and butyrylthiocholine hydrolysis is more than other substrates. At the same time, this type of new enzyme differs from other typical BuChE, so the hydrolysis rate of butyrylcholine by FChE is in 10 - 13 times more rapid as compared to hydrolysis of acetylcholine. The sensitivity of FChE to organophosphates is in 100-1200 times higher than the sensitivity of all types of commercial ChE. It is very unexpectedly and unordinary that FChE has a very small sensitivity to active carbamates and reversible ChE inhibitors. The results obtained in this investigation suggested that there might be an essential difference between the active sites of FChE and another types of cholinesterases.

LETHAL EFFECTS OF HEAD-TO-TAIL 3-ALKYLPYRIDINIUM POLYMERS ISOLATED FROM THE MARINE SPONGE RANIERA SARAI: AChE INHIBITION OR UNSPECIFIC BINDING TO SERUM PROTEINS?

M. Bunc¹, K. Sepcic², A. Rotter¹, T. Turk², A. Vidmar¹, D. Suput¹

¹Medical Faculty, Institute of Pathophysiology, ²Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

The toxic aqueous extract polymeric 3-alkylpyridinium salts were isolated from the marine sponge *Raniera sarai*. Both in vitro and in vivo experiments were performed to evaluate the role of acetylcholinesterase (AChE) inhibition in the toxin lethality. Binding of the polymer to the *Drosophila* AChE was estimated by the fluorescence of the protein polymer complex (excitation at 290 nm). On male Wistar rats ECG, blood pressure and breathing pattern were monitored. In vitro the polymers show a quick initial reversible binding, followed by slow binding and finally by irreversible inhibition of AChE. In vivo doses lower than 1 mg/kg caused bradycardia and prolongation of expiration. At doses above 2.7 mg/kg all treated animals died, but signs were not typical of acetylcholinesterase inhibition. Binding of the *R. sarai* inhibitor seems to occur at the active site gorge. However, unusual inhibition kinetics suggests that there are several lower affinity binding sites. At lower doses, the toxin caused changes of the measured parameters typical of AChE inhibition. At lethal doses the possible AChE inhibitory effects were probably covered by the other, more pronounced lethal effects of the toxin. At both concentrations only small fraction of the toxin could act in neuromuscular junction since the huge molecular conglomerates (20 nm) of the toxin molecules develop in vitro. Those aggregates could hardly cross the capillary wall and enter the neuromuscular junction in vivo. The influence of the toxin on platelets aggregation, and later fibrin segregation could be the primary and most important in the toxin lethality.

STRUCTURAL INSIGHTS INTO THE INTERACTIONS AT THE ACETYLCHOLINESTERASE PERIPHERAL ANIONIC SITE

Y. Bourne¹, P. Taylor², H.A. Berman³, Z. Radic², P. Marchot⁴

¹AFMB CNRS, Marseille, France, ²Department of Pharmacology, UCSD, La Jolla, CA, USA, ³Department of Biochemical Pharmacology, School of Pharmacy, SUNYB, Buffalo, NY, USA, ⁴CNRS, Institut Federatif de Recherche Jean Roche, Université de la Méditerranée, Marseille, France

The AChE peripheral anionic site, located at the entrance of the enzyme active site gorge, consists of a matrix of overlapping loci for the binding of the diverse ligands that serve as allosteric activators or inhibitors; yet, the molecular mechanisms coupling this site to the remote active center to modulate catalysis remain unclear. The peripheral site has also been proposed to be involved in cellular adhesion through formation of heterologous protein interactions that occur during synaptogenesis or the onset of degenerative neuropathologies. Based on a new crystal form of mouse AChE and combined spectrophotometric crystal analyses, unique structures of the enzyme with a free peripheral anionic site, and as three complexes with phenanthridinium and pyrogallol peripheral site inhibitors, were solved in the 2.20-2.35 Å resolution range. A comparative analysis with earlier structures of AChE in complexes with the peptide fasciculin or with organic bifunctional inhibitors unveils new structural determinants contributing to ligand interactions at the AChE peripheral anionic site, and permits a detailed topographic delineation of its locus. Hence, these structures provide templates for ligand docking that may be useful for designing compounds directed to the enzyme surface and able to modulate specifically surface interactions involved in non-catalytic AChE function. Supported by USPHS grant GM18360 and DAMD grant 17-1-8014 to PT; the NSF-CNRS collaborative project #3906 to PM and PT; the AFM to PM and PE Bougis (CNRS-UMR6560).

PIPERONYL BUTOXIDE: A SPECIFIC INHIBITOR OF INSECTICIDE RESISTANT ACETYLCHOLINESTERASE

R.V. Gunning

NSW Agriculture, Tamworth, NSW, Australia

The methenedioxyphenyl compound piperonyl butoxide (PBO) has a long history as an insecticide synergist, yet it is a compound whose mode of action is still poorly understood. PBO was long assumed to be solely a cytochrome P450, however, it is known to be capable other functions, such as esterase inhibition. The objective of this study was to explore to effects of PBO on insect acetylcholinesterase (AChE). In vitro, PBO inhibited organophosphate or carbamate insensitive AChE in *Helicoverpa armigera*, *Helicoverpa punctigera*, B-biotype *Bemisia tabaci* and *Aphis gossypii*. Inhibition was particularly strong in *H. armigera*. In vivo studies also supported these findings. PBO appeared to enhance the activity of insecticide sensitive AChE (*H. armigera*, *H. punctigera*, *B. tabaci*, *A. gossypii*), however, this is likely consequence of PBO interaction with the substrate acetylthiocholine iodide. Inhibition of insensitive AChE provides very exciting possibilities for the control of an intractable resistance mechanism. While PBO is unlikely to be acting as a conventional inhibitor of insensitive acetylcholinesterase, observations suggest that PBO is entering a physicochemical reaction with insensitive AChE, making it unavailable to the substrate. While it is unknown, as to how structure differs between sensitive and insensitive AChE, it is commonly the case that the mutation conferring insensitivity decreases the affinity of the protein for its natural substrate. PBO may have a three dimensional structure able to penetrate the AChE active site.

SIGNIFICANCE OF PARAMETERS BETWEEN VARIOUS KINETIC SCHEMES FOR CHOLINESTERASES

J. Stojan, M. Golcnik

Institute of Biochemistry, Medical Faculty, University of Ljubljana, Slovenia

Cholinesterases show homotropic pseudo-cooperative phenomena. At intermediate substrate concentrations the activities of these enzymes exceed the expected values according to Michaelis-Menten mechanism and at very high substrate concentrations they are inhibited. A kinetic model [Stojan et al. FEBS Lett. 440 (1998) 85-88] was put forward which can describe the data of entire substrate concentration interval and also in the presence of the inhibitors. However, the criticism has been raised, since some steps in the reaction mechanism were roled together, thus not reflecting subsequent events during the catalytic process. Although some evaluated constants can be explained in terms of classical kinetic parameters, we performed an extensive mathematical characterisation to evaluate the significance of determined kinetic parameters. We have compared their values and derived the appropriate connections with Webb model and with the traditional reaction scheme for vertebrate acetylcholinesterase.

STUDIES OF ACETYLCHOLINESTERASE FROM THE PEACH-POTATO APHID, MYZUS PERSICAE(SULZ.)

N. Javed¹, M.S. Williamson¹, A.L. Devonshire¹, R.C. Viner², T. Lewis², G.D. Moores¹

¹IAC'R-Rothamsted, Harpenden, Herts, ²Syngenta, Jealotts Hill International Research Centre, Bracknell, UK

Acetylcholinesterase (AChE) is the target site for two important classes of insecticides, organophosphates (OPs) and carbamates. Widespread use of these compounds has led to the development of resistance in many insect species, often due to an insensitive form of the enzyme. To identify the structural changes that confer pirimicarb and triazamate insensitivity to acetylcholinesterase (AChE) in the peach-potato aphid, *Myzus persicae*, the ace gene (600 amino acids) from susceptible and resistant clones were sequenced using RT-PCR and RACE. However, no mutational changes were identified between the sensitive and insensitive enzymes. Possible explanations for this observation include that of post-translational modification, or the possibility that the sequence obtained is not that of the synaptic AChE. Since primers used were derived from conserved regions of dipteran sequences, novel reversible inhibitors synthesised by Syngenta were employed to elucidate possible differences between sternorrhyncha and non- sternorrhyncha species. Multidimensional scaling and hierarchical cluster analysis of inhibition data utilising these reversible inhibitors against a variety of insect species revealed compelling evidence that the sequence obtained was not that of synaptic AChE.

EXPLORING THE ACHE GORGE WITH GALANTHAMINE

G. Fels, E. Linnemann, E. Luttmann, C. Pilger

University of Paderborn, Germany

Acetylcholinesterase (AChE) plays a key role in the development of Alzheimer's disease as this enzyme is responsible for cleavage of the neurotransmitter acetylcholine (ACh), and, according to recent investigations, also promotes aggregation of beta-amyloid peptides, which causes plaque formation in synaptic areas. While the catalytic triad responsible for ACh-cleavage is located 20 Angstrom deep in the enzyme at the end of narrow gorge, the beta-amyloids seem to attach to the peripheral anionic site (PAS) centered around amino acid Trp279 at the mouth of the gorge. We have investigated the potential of galanthamine, and some derivatives of this alkaloid with varying N-substituents, to interfere with both these functions in the AChE-gorge. Our studies result in a correct prediction of the orientation and conformation of galanthamine in the active site of AChE from *Torpedo californica* (TcAChE) with a RMS deviation of about 0.5 Angstrom with respect to the crystal structure of the TcAChE-galanthamine complex. The docking studies, furthermore, reviled a second binding site for galanthamine which is located at the PAS. Based on these findings, we have performed a molecular modeling study to investigate bis-galanthamine derivatives that have two galanthamine moieties connected by a methylene spacer of varying length as dually acting AChE-ligands. Our results propose that such ligands indeed can simultaneously interfere with both biological functions of the enzyme, the neurotransmitter cleavage and the plaque formation and should therefore serve as the basis for a further development of bis-functional Alzheimer drugs.

HYSTERESIS IN BUTYRYLCHOLINESTERASE CATALYSIS: EVIDENCE FOR SUBSTRATE-INDUCED CONVERSION OF THE ENZYME FROM LATENT TO OPERATIVE FORM

P.-Y. Masson¹, M.-T. Froment¹, F. Nachon¹, L.M. Schopfer²

¹CRSSA, La Tronche, France, ²University of Nebraska Medical Center, Eppley Institute, Omaha, NE, USA

Human butyrylcholinesterase (BuChE, EC. 3.1.1.8) was found to reach slowly steady-state velocity with the neutral ester N-methylindoxyl acetate as substrate. The lag rate decreased with increasing substrate concentration. The kinetic mechanism of this hysteretic process was interpreted in terms of slow conformational change of the enzyme from an inactive to an active form that binds and hydrolyzes substrate [1]. Hysteresis by peripheral site mutants (D70G, Y332A, D70G/Y332A) was similar to that of wild-type enzyme. In addition, hysteresis was shown for hydrolysis of positively charged substrates: benzoylcholine with wild-type; butyrylthiocholine with the D70H mutant and different active site mutants, e.g., A328C and G117H/E197Q. This clearly indicated that hysteresis is a general property of BuChE which does not involve the peripheral site. Resolution of the x-ray structure of BuChE provided evidence that the native BuChE is a 'dormant' enzyme [2], and Nicolet et al., this symposium]. Therefore, lags in progress curves for substrate hydrolysis can be interpreted as the time needed to convert the whole population of dormant enzyme forms to an operative one, performing catalytic turnover at steady-state velocity. A new mechanistic model for catalysis of BuChE is proposed. [1] P. Masson, M.T. Froment, S. Fort, F. Ribes, N. Bec, C. Balny and L.M. Schopfer, Butyrylcholinesterase-catalyzed hydrolysis of N-methylindoxyl acetate: analysis of volume changes upon reaction and hysteresis behavior, *Biochim. Biophys. Acta* (2002) in press. [2] F. Nachon, Y. Nicolet, N. Viguie, P. Masson, J.C. Fontecilla-Camps and O. Lockridge, Engineering of a monomeric and low-glycosylated form of human butyrylcholinesterase: expression, purification, characterization and crystallization, *Eur. J. Biochem.* (2002) 269, 630-637.

A CALCIUM-PROTON ANTIPORT IN CHOLINERGIC AND GLUTAMATERGIC SYNAPTIC VESICLES

M. Cordeiro, V. Bancila, A. Bloc, Y. Dunant

Departement de Pharmacologie, CMU, Geneva, Switzerland

Neurotransmission in rapid synapses implies reactions where 'time is gained at the expense of sensitivity' (Katz, 1988). Indeed, bursting of nicotinic or glutamate AMPA receptors typically occurs in response to a brief exposure to relatively high transmitter concentrations; desensitisation occurs if exposure to the ligand is prolonged. Similarly, quantal transmitter release, supported by a plasmalemma proteolipid (mediatophore), is activated by a brief and high local increase of Ca^{2+} concentration; and also desensitises if Ca^{2+} is not rapidly removed. Calcium has long been known to be transported into synaptic vesicles and reticulum by a Ca^{2+} -ATPase, a high affinity system working rather slowly. In addition, a $\text{H}^{+}/\text{Ca}^{2+}$ antiport has recently been demonstrated to operate in synaptic vesicles of CNS cortex. We are investigating the physiological role of the $\text{H}^{+}/\text{Ca}^{2+}$ antiport in the mammalian brain and Torpedo electric organ by using either intact synapses, or synaptosomes or isolated vesicles. Our and other data support the following sequence of events in presynaptic nerve terminals submitted to intense stimulation 1) Ca^{2+} concentration abruptly rises in limited microdomains under the presynaptic membrane, activating mediatophores and triggering release; 2) The local Ca^{2+} concentration drops rapidly in a first phase ($\text{H}^{+}/\text{Ca}^{2+}$ antiport) and then more slowly ($\text{Na}^{+}/\text{Ca}^{2+}$ antiport, Ca^{2+} ATPase, and other buffering processes); 3) Ca^{2+} accumulation in synaptic vesicles causes neurotransmitter leakage from the organelle, fuelling the local pool available for release; 4) Accumulated Ca^{2+} is expelled to synaptic cleft by exocytosis.

Supported by the FNRS Grant No 31 57135 99 (Switzerland) and SFRIIBD64032001 (Portugal)

OXACHEIN, A NOVEL POTENT INHIBITOR OF ACETYLCHOLINESTERASE FROM A PLANT-- OXALIS CORNICULATA L.

R. Gupta, A. Gupta

Physiology and Medicine Laboratory, Department of Botany, University of Delhi, India

Anticholinesterases (antiChE) have been used as medicines, pesticides and agents of chemical warfare. A variety of natural and synthetic antiChE are known. However, the search is on for new and more effective antiChE. We report a novel and potent antiChE compound from roots of a plant, *Oxalis corniculata* L. and name it as oxachein (*Oxalis* ChE inhibitor). Oxachein has dull white needle shaped crystals having melting point 145-145.2°C. Based on data obtained by employing NMR, MS, X-ray diffraction and other analytical techniques, oxachein is 2-(3,5-dimethoxyphenyl)-5-(3,5-dihydroxyphenyl)-6-(4-hydroxyphenyl)-5,6-dihydro-furo-(3,2-f)benzofuran (MW = 496). To the best of our knowledge, this compound has neither been synthesized nor reported from any natural source. Oxachein is a very potent inhibitor of AChE (EC 3.1.1.7) from electric eel (50 percent inhibition at 0.8 microM, 100 percent inhibition at 5 microM). However, it is a weak inhibitor of ChE from roots of a plant *Cicer arietinum* L. (chickpea) causing only 23 percent inhibition at a concentration of 10 microM. Lineweaver-Burk plot study shows non-competitive mode of inhibition of eel AChE. Unlike some other antiChEs from plants, oxachein is neither an alkaloid nor a terpenoid. It may serve as a prototype for novel antiChEs in drug development programmes.

(With inputs from Prof. M.R. Parthasarathi, Department of Chemistry, University of Delhi, Delhi and Prof. T.P. Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi. Supported by a U.G.C. research grant to RG and fellowship from CSIR to AG).

MECHANISM OF ACETYLCHOLINESTERASE INHIBITION BY FASCICULIN: A 5 NANOSECOND MOLECULAR DYNAMICS SIMULATION

K. Tai¹, T. Shen¹, R.H. Henchman¹, Y. Bourne², P. Marchot³, J.A. McCammon⁴

¹University of California, San Diego, La Jolla, CA USA, ²Institut de Biologie et Microbiologie Structurale, Marseille, France, ³Universite de la Mediterranee, Marseille, France, ⁴Howard Hughes Medical Institute and University of California, San Diego, La Jolla, CA, USA

In our previous molecular dynamics simulation of mouse acetylcholinesterase (EC 3.1.1.7), the enzyme revealed complex gorge fluctuation. Now we report another 5 ns simulation, with acetylcholinesterase complexed with fasciculin 2. Fasciculin 2 binds to the gorge entrance of acetylcholinesterase with excellent complementarity and many polar interactions. In this simulation of the protein-protein complex, we continue to observe a two-peaked probability distribution of the gorge width, though fasciculin 2 appears to block access of ligands to the gorge. The gorge width distribution is altered when fasciculin is present: the gorge is more likely to be narrow. Though the gorge is sterically blocked by fasciculin 2, there are large increases in the opening of alternative passages, namely the side door and the back door. The catalytic triad arrangement in the acetylcholinesterase active site is disrupted with fasciculin bound. These data suggest that, in addition to the steric obstruction, fasciculin may inhibit acetylcholinesterase by allosteric and dynamical means. Additional data from these simulations can be found at <http://mccammon.ucsd.edu/>.

PROF. RENE COUTEAUX AND HIS PRESYNAPTIC "ACTIVE ZONE"

Shigeru Tsuji

Department of Cytology, Institute of Neuroscience, Pierre et Marie Curie University, Paris, France

Couteaux had a notion of the active zone around 1961. He wrote his idea only as a personal communication to J. Eccles in 1961. Eccles in "The Physiology of Synapses" (1964) cited the term of Couteaux erroneously from one of his publications in 1961. To my sticky question for the historical background, Couteaux wrote his answer on a piece of paper in 1997 (Fig. 1). Indeed, the active zone was used in public for the first time in an article of Couteaux R. and Pécot-Dechavassine M. (CR Acad Sci Paris 1970, 271, 2346). We read there "What we call hypothetically the active zones". Couteaux had not claimed paternity of the term. He knew that similar ideas existed already, though electron microscopic proof was not available. Couteaux needed absolutely an image of the exocytosis of synaptic vesicles from a specialized dense part of the presynaptic membrane of the motor nerve terminal. For this purpose, he spent almost ten years.

MITOGEN-ACTIVATED PROTEIN KINASE KINASE INHIBITS CILIARY NEUROTROPHIC FACTOR-ACTIVATED CHOLINE ACETYLTRANSFERASE GENE EXPRESSION

T. Mellott¹, I. Lopez-Coviella², J.K. Blusztajn¹, B. Berse¹

¹Department of Pathology, ²Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA

The expression of choline acetyltransferase (ChAT), which synthesizes acetylcholine (ACh) is upregulated by ciliary neurotrophic factor (CNTF). We investigated the role of the mitogen-activated protein kinase (MAPK) pathway in regulating CNTF-stimulated ChAT expression. Using the murine septal cell line SN56T17, we found that PD98059 and U0126, two structurally distinct inhibitors of MAPK kinase (MEK1), increased both basal and CNTF-stimulated ACh production. Transient transfections with ChAT promoter-luciferase reporter construct demonstrated synergy between PD98059 and CNTF at the transcriptional level. Moreover, in co-transfection studies, overexpression of constitutively active MEK1 completely inhibited the CNTF-mediated induction of the cholinergic promoter. To elucidate the mechanism of this inhibition, we examined the signaling pathways evoked by CNTF in SN56T17 cells. Tyrphostin AG-490, a Jak2 inhibitor, abrogated both the activation of the transcription factor Stat3 (measured as Tyr705 phosphorylation) and the increase in ACh production stimulated by CNTF, indicating that Stat3 is a mediator of the CNTF effect. PD98059 did not significantly alter CNTF-induced Tyr705 phosphorylation of Stat3, but it inhibited Ser727 phosphorylation, demonstrating that the latter is MEK1-dependent. The results indicate that MEK1 inhibits the CNTF-mediated stimulation of ChAT expression, possibly as a part of a feedback mechanism.

PLANT CHOLINESTERASE ACTIVITY AS A BIOSENSOR FOR TOXINS IN THE ENVIRONMENT

Victoria V. Roshchina

Institute of Cell Biophysics RAN, Pushchino, Moscow, Russia

Plant cholinesterases are sensitive to derivatives of carbaminic acid-neostigmine and physostigmine and organophosphorus compounds-diisopropyl phosphorofluoridate, dichlorvos, phosphon and quaternary ammonium compounds as many other artificial and natural toxins (Roshchina, 2001). Cholinesterase activity of pollen *Hemerocallis fulva*, *Tulipa sp.*, *Salix caprea*, *Allium cepa*, and *Hippeastrum hybridum* was studied with Ellman reagent and its new red azoanalog as biotest on the pesticides - dichlorvos, rigor and some synthesized organophosphates GD-42 and GD-7, carbamates neostigmine and its derivatives and plant toxins physostigmine, allicin, atropine, solanine (Roshchina, 2001). There are differences between the pollen sensitivity to physostigmine and neostigmine for certain species, for instance, more lower to first inhibitor for *Hippeastrum* and *Salix* pollen. If to compare I_{50} for carbamate inhibitors between cholinesterases from plant and animal tissues, pollen cholinesterases seem to be most sensitive among the plant enzymes. Organophosphates demonstrate lower specificity to cholinesterases than carbaminic inhibitors. Cholinesterase of anther of *Hippeastrum* is sensitive to only organophosphate GD-42, but not to GD-7 or iso-OMPA. Natural toxins, except atropine, inhibited the plant cholinesterase with the same degree as carbamates. Pollen cholinesterase can be used as biosensor on the toxins of the environment.

Reference: Roshchina V.V. (2001) *Neurotransmitters in Plant Life*. Einfield, New Hampshire, New Delhi: Science Publishers and Oxford Publishing Co, 289 pp

EFFECTS OF METHOMYL ON SPLEENS AND APOPTOSIS

T. Posayanonda¹, T. Suramana¹, N. Nuntharatanapong¹, O. Lohitnavy¹, R. Snowden², W. Schwachble², N. Dusitsin³, R. Sindhupak³, P. Sinhaseni¹

¹Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, ²University of Leicester, UK, ³Institute of Health Research Chulalongkorn University, Bangkok, Thailand, ⁴Naresuan University, Pitsamulok, Thailand

Methomyl, a carbamate insecticide widely used for crop protection, is known as an acetylcholinesterase inhibitor. Its reported toxic effects are mostly cholinergic effects. However, some toxic effects reported from methomyl may not be related to the anticholinesterase action. Our studies reported that 24 hour-single dose exposure of 8 mg/kg methomyl po. caused a significant decrease in rat splenocyte viability, induced oxidative stress, and showed the conformational change of proteins at amide-I and -II regions in rat spleen cells. However, effect of methomyl on protein conformation in spleens could not be blocked by anticholinergic agent, atropine. Therefore, toxicity of methomyl could be also generated by mechanisms other than acting through acetylcholinesterase and acetylcholine. Effects of methomyl on leukocytic cells have been studied. The results showed that 6 hour-exposure of methomyl could reduce mitochondrial transmembrane potential and induce caspase-dependent apoptosis in MM6, THP-1, and Jurkat cell lines at final concentrations of 18, 18, and 6 mM, respectively. Its action on IL-6 signaling pathway was found to play roles. Methomyl did not induce apoptosis in Raji cells, but caused a cell cycle arrest in the G0/G1 phase. The ability of methomyl to induce protein conformational change in spleens and apoptosis in the leukocytic cell lines is probably generated from its action as a sulfhydryl binding agent, which can interfere with intracellular signals. The study of effects of methomyl on signal transduction alterations will provide more information of mechanisms of methomyl toxicity.

EFFECTS OF CARBAMATE INSECTICIDES ON RAT NEURONAL $\alpha 4\beta 4$ NICOTINIC RECEPTORS AND RAT BRAIN ACETYLCHOLINESTERASE

C.J.G.M. Smulders¹, T.J.H. Bueters², H.P.M. Vijverberg¹

¹Institute for Risk Assessment Sciences, Utrecht University, Utrecht, ²TNO PML, Research Group Pharmacology, Rijswijk, The Netherlands

Carbamate insecticides are generally known to inhibit the enzyme acetylcholinesterase (AChE). In addition, some carbamates, e.g. physostigmine, modulate neuronal nicotinic ACh receptor (nAChR) function by potentiating the ACh-induced ion current. The aim of this study was to investigate the effects of carbamate insecticides on nAChRs and to compare these effects with those on AChE. Rat $\alpha 4\beta 4$ nAChRs, expressed in *Xenopus laevis* oocytes, were exposed to different concentrations of insecticides. ACh-evoked currents were measured using a two-microelectrode voltage clamp. Unlike physostigmine, all 6 carbamates tested inhibited 1 mM or 1 μ M ACh-induced ion currents. Potencies ranged from 5 μ M for fenoxycarb to 1.2 mM for aldicarb. Inhibition of rat brain AChE activity was determined spectrophotometrically for all compounds. Fenoxycarb and EPTC caused no inhibition, whereas bendiocarb and propoxur proved to be the more potent inhibitors of rat brain AChE with potencies of 0.75 μ M and 2.4 μ M, respectively. The results demonstrate that the potentiating activity of physostigmine does not solely depend on the presence of a carbamate moiety. There is no correlation observed between inhibition of the rat $\alpha 4\beta 4$ nAChR and inhibition of rat brain AChE. Some carbamates are more potent inhibitors of the rat $\alpha 4\beta 4$ neuronal nAChR than of rat brain AChE, suggesting that neuronal nAChRs are an additional target for carbamate insecticide neurotoxicity.

**SITE-SPECIFIC ANALYSIS OF GLYCAN STRUCTURES ON
PLASMA-DERIVED HUMAN (Hu) AND HORSE (Eq)
BUTYRYLCHOLINESTERASES (BChE)**

G.E. Garcia¹, D.R. Moorad-Doctor¹, O. Lockridge², C.B. Millard³,
C.A. Broomfield¹, B.P. Doctor¹, A. Saxena¹

¹Department of Biochemistry, WRAIR, Silver Spring, MD, USA, ²Eppley Cancer
Institute, University of Nebraska Medical Center, Omaha, NE, USA, ³US Army
Medical Research Institute of Infectious Diseases,
Fort Detrick City, Frederick, MD, USA

The successful use of plasma-derived cholinesterases as pretreatment drugs for organophosphate toxicity, stems from their long mean retention times. The relatively high stability of these enzymes in circulation may be attributed to the number and structures of their carbohydrate residues. Therefore, the site-specific carbohydrate (CHO) structures of several soluble BChEs were determined. Purified proteins were fragmented by enzymatic or chemical means. Glycopeptides were purified by reverse-phase HPLC and identified by N-terminal sequencing. Asparagine-linked glycans were released by treatment with PNGase F. The free reducing ends were labeled with 8-aminonaphthalene-1,3,6-trisulfonate (Glyko, Inc.), and subjected to fluorophore-assisted carbohydrate electrophoresis analysis following sequential digestion with various glycosidases. Site-specific CHO structures were determined for 8/9 Hu and 3/8 Eq BChEs. For Hu BChE, the major structure consisted of an asialyloligosaccharide without core fucosylation. Hu BChE site 8 (aa#481) and 9 (aa#486) were inseparable with the cleavage strategies employed, which precluded an absolute assignment of CHOs for these sites. Glycans of Eq BChE, were similar except that the sites displayed less microheterogeneity and the majority of structures were sialylated. While all the sites for Hu BChE displayed some degree of microheterogeneity, some sites were more heterogeneous; for example site 4 (aa#241) had 25% oligo-mannose structures, while sites 5 (aa#256) and 7 (aa#455) consisted of 11% and 12% tri-galactose, tri-antennary structures, respectively. The notable lack of sialylation of Hu BChE CHOs was probably due to the prolonged storage of the glycopeptides. CHO structures for fewer Eq BChE sites were determined, but a direct comparison (Hu site 3 and Eq site 2) indicated a high degree of homology between them.

**MECHANISM AND STRUCTURAL REQUIREMENTS OF
XANOMELINE WASH-RESISTANT BINDING TO M1
MUSCARINIC RECEPTORS**

J. Jakubik¹, E. El-Fakahany², S. Tucek¹

¹Institute of Physiology, Academy of Sciences, Prague, Czech Republic,

²Neuroscience Research in Psychiatry, University of Minnesota,
Minneapolis, MN, USA

Xanomeline is a novel agonist functionally selective for muscarinic M1 receptors. It is unusual in that it binds to receptors both in a reversible manner (with submicromolar affinity) and in a wash-resistant, quasi-irreversible manner (with micromolar affinity). We investigated the two modes of its binding on membranes from CHO cells expressing M1 muscarinic receptors, with the following conclusions:

(1) Reversible binding of xanomeline occurs at the classical binding site and is competitive with that of classical muscarinic ligands. (2) Stable binding (half-life of >24 h) is to an "exosite", distinct from the classical site. (3) Stable binding to the exosite is preceded by initial high-affinity binding, but an interaction of xanomeline with the classical binding site is not a prerequisite for its binding to the exosite. (4) Comparison of xanomeline with its analogues indicates that the wash-resistant binding of xanomeline depends on the presence of the O-hexyl arm in its molecule and that an at least five-carbon long O-alkyl chain is required for wash-resistant binding.

The length of the O-alkyl chain required and thermodynamic data suggest that the wash-resistant binding depends on the penetration of the O-alkyl chain of xanomeline between individual alpha-helices of the binding pocket and its direct interaction with lipids surrounding the receptor.

**RECOVERY FROM DESENSITIZATION OF A NEURONAL
NICOTINIC RECEPTOR**

S. Voytenko¹, R.J. Lukas², R. Gruener¹

¹Department of Physiology, University of Arizona, Tucson, AZ, USA

²Barrow Neurological Institute, Phoenix, AZ, USA

We have shown (J. Pharm. Exptl. Ther. 289:656,1999) that skeletal muscle nicotinic acetylcholine receptors (nAChR) recover from desensitization depending on concentration and duration of exposure to agonist and its identity. Recovery after ACh exposure was significantly slower than that induced by nicotine (Nic) for all concentrations. Further, the muscle type nAChR displays a 'molecular memory' probably resulting from the conformational state that the receptor undergoes in response to agonist-specific desensitization.

Using whole cell patch voltage-clamping (EPC9) combined with rapid agonist delivery (Warner Instruments, Fast-Step), we examined responses to ACh and Nic in SHSY-5Y cells expressing the ganglionic, neuronal nAChR ($\alpha 3 \beta 4$; $\alpha 5 \beta 2$). Our data indicate that recovery from desensitization induced with ACh or Nic proceeds in a double exponential fashion except for the shortest durations of exposure we used (1 s or less) in which recovery kinetics were best fit with a single exponential. In all cases, recovery from desensitization proceeded considerably faster when ACh induced desensitization. Further, when ACh was used to desensitize and Nic to measure recovery, the kinetics of recovery were similar to those found when Nic alone was used to measure responses. Similarly, when Nic was used to desensitize and ACh to monitor recovery, kinetics were similar to those where cells were exposed to ACh alone.

We conclude that the neuronal receptor examined here behaves differently from the muscle type with respect to desensitization and recovery and that this difference is consistent with the known addictive effects of nicotine on neuronal cholinergic receptors.

**IS THE G-PROTEIN-COUPLED M2 MUSCARINIC RECEPTOR A
VOLTAGE SENSOR?**

O. Tour¹, N. Dascal², Y. Ben Chaim³, I. Parnas³, H. Parnas³

¹Department of Pharmacology, University of California San Diego, La Jolla, CA, USA, ²Department of Physiology and Pharmacology, Tel Aviv

University, Israel, ³The Otto Loewi Minerva Center for Cellular and
Molecular Neurobiology, The Hebrew University of Jerusalem, Israel

G-protein coupled receptors (GPCRs) are not considered to exhibit voltage sensitivity. Here, using *Xenopus* oocytes, we examined whether a classical GPCR, the muscarinic M2-receptor (m2R), is by itself a voltage sensor. Oocytes expressing m2R were voltage-clamped at -60 mV or at +40 mV, and the relationship between the G-protein-gated inwardly rectifying K⁺ channels (GIRK) response and acetylcholine (ACh), (a positively charged agonist) or oxotremorine (OXO), (an uncharged agonist), concentration was established at these two holding potentials. We found, for both agonists, that at +40 mV there is a shift to the right of the dose response curve. Direct binding experiments of [3H]-ACh to individual oocytes expressing m2R showed that the specific binding of [3H]-ACh was reduced by depolarization. These results suggest that the m2R senses changes in membrane potential.

SOME BASIC RULES GOVERNING OLIGOSACCHARIDE-DEPENDENT CIRCULATORY RESIDENCE OF GLYCOPROTEINS ARE REVEALED BY MALDI-TOF MAPPING OF THE MULTIPLE N-GLYCANS ASSOCIATED WITH RECOMBINANT BOVINE ACETYLCHOLINESTERASE

C. Kronman¹, T. Chitlaru¹, N. Seliger¹, S. Lazar¹, A. Lazar², L. Zilberstein², B. Velan¹, A. Shafferman¹

¹Department of Biochemistry and Molecular Genetics, ²Department of Biotechnology, Israel Institute for Biological Research, Ness-Ziona, Israel

Pharmacokinetic studies of recombinant bovine acetylcholinesterase (rBoAChE), revealed that this enzyme is cleared more rapidly than the native serum-derived FBS-AChE. Extensive MALDI-TOF analysis of sialylated and desialylated glycans purified from rBoAChE, revealed that these are comprised of a complex array of diverse structures differing in branching, monosaccharide substitutions and relative abundances. The exact structures of the different glycans were confirmed by a series of exoglycosidase treatments followed by MALDI-TOF analysis. The most prevalent structure was the biantennary fucosylated form, (Man)₃-(GlcNAc)₄-(β -gal)₂-Fuc, which constitutes approximately 40-50 percent of the total glycans. 20-30% of the glycans were of the triantennary form, while tetraantennary glycans were present at very low levels. Most importantly, the glycans of rBoAChE were found to be heavily undersialylated, containing ~4.5 terminally exposed β -gal per enzyme subunit.

To allow efficient sialylation, rBoAChE was produced in an engineered HEK-293 cell line clone which expresses high levels of recombinant sialyltransferase. MALDI-TOF analysis of the glycans of rBoAChE produced in these cells demonstrated that the vast majority of these glycan forms were now highly sialylated. Pharmacokinetic studies of highly-sialylated rBoAChE established that this enzyme was retained in the circulation for extended periods of time, as compared to undersialylated rBoAChE. These studies, emphasize the pivotal role of glycan sialylation in determining the circulatory fate of cholinesterases, and provide the basis for detailed determination of their glycan structures.

This work was supported in part by the U.S. Army Research and Development Command, Contracts DAMD17-96-C6088 and DAMD17-00-C0021 (to A.S.)

CHANGES IN NEURONAL CHOLINERGIC RECEPTOR BINDING SITES AT DIFFERENT AGES IN TRANSGENIC MICE OVEREXPRESSING HUMAN ACETYLCHOLINESTERASE

M.M. Svedberg, A.-L. Svensson, I. Bednar, A. Nordberg

Division of Molecular Neuropharmacology, NEUROTEC, Karolinska Institutet, Stockholm, Sweden

Deficits in the cholinergic circuits of the human brain are observed in neurodegenerative disorders like Alzheimer's and Parkinson's disease. An overexpression of neuronal human acetylcholinesterase (AChE) in mice causes alteration in the cholinergic transmission. The objective of this study was to investigate how an overexpression of AChE activity influence the plasticity of cholinergic neurons, particularly the nicotinic and muscarinic receptor subtypes. AChE transgenic (Tg+) mice at different ages, from 3 days old up to 1 year, were compared to age-matched non-transgenic (Tg-) mice. The nicotinic receptor binding sites were quantified, in the cortex and the striatum using [³H]cytisine (alpha4beta2) and in the cortex and the hippocampus using [¹²⁵I]alpha-bungarotoxin (alpha7). In addition, muscarinic receptor binding sites were quantified, in the cortex and the striatum using [³H]AF-DX-384 (M2) and in the cortex using [³H]pirenzepine (M1). A significantly increased [³H]cytisine binding were found in the cortex and the striatum in AChE Tg+ mice in comparison to Tg- mice in various age groups. No major alteration in [¹²⁵I]alpha-bungarotoxin binding sites were observed in Tg+ compared to Tg- mice. However, an up-regulation of [³H]AF-DX-384 binding sites were found in the striatum in AChE overexpressing mice at 3 month of age compared to age-matched control mice while no change in [³H]pirenzepine binding sites were detected at any age. The increase in alpha4beta2 and M2 receptor binding sites observed in AChE Tg+ mice was found at all ages and thus not influenced by aging processes.

EFFECT OF POST-TRANSLATION MODIFICATIONS OF HUMAN ACETYLCHOLINESTERASE ON ITS CIRCULATORY RESIDENCE

T. Chitlaru, C. Kronman, S. Lazar, N. Seliger, B. Velan, A. Shafferman

Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness Ziona, Israel

Homogenous preparations of rHuAChE differing in their oligomerization status were generated: (a) monomers - represented by the oligomerization-impaired C580A-rHuAChE mutant (b) dimeric wild-type (WT) and (c) tetramers of WT-rHuAChE generated *in-vitro* by complexation with a synthetic ColQ-derived Proline Rich Attachment Domain (PRAD) peptide. Three different series of each of these three oligoform preparations were produced: 1) partially sialylated - derived from HEK-293 cells; 2) fully sialylated - derived from HEK-293 engineered cells expressing high levels of sialyltransferase; 3) desialylated - following treatment with sialidase to quantitatively remove sialic acid termini. The oligosaccharides associated with each of the various preparations were extensively analysed by MALDI-TOF. With the enzyme preparations comprising the fully sialylated series, a clear linear relationship between oligomerization and circulatory mean residence time (MRT) was observed. Thus, monomers, dimers and tetramers exhibited MRTs of 110, 195, and 740 min. respectively. As the level of sialylation decreased, this differential behavior became less pronounced, and eventually following desialylation all oligoforms had the same MRT (5 min). These observations suggest that multiple removal systems contribute to the elimination of acetylcholinesterase from the circulation. The studies presented here demonstrate also that by combined modulation of sialylation and tetramerization it is possible to generate a rHuAChE displaying a circulatory residence exceeding that of all other known forms of native or recombinant human AChE.

This work was supported in part by the U.S. Army Research and Development Command, Contract DAMD17-00-C0021 (to A.S.)

TRANSGENIC OVEREXPRESSION OF READTHROUGH ACETYLCHOLINESTERASE (ACHE-R): DISTRIBUTION OF ACHE-R AND CFOS IN BRAIN IN RELATION TO BEHAVIOR

S. Shoham¹, O. Cohen², S. Dishon², R. Yirmiyah³, E. Kovalev¹, D. Ginzberg², H. Soreq²

¹Research Department, Herzog Hospital, Jerusalem, ²Biological Chemistry, Hebrew University, Jerusalem, ³Psychology Department, Hebrew University, Jerusalem, Israel

Acute stress induces a cascade of events, which involves increased release of acetylcholine and feedback activation of acetylcholinesterase gene expression, shifting into the "readthrough" acetylcholinesterase variant AChE-R which contributes to downregulating stress-induced cholinergic excitability. To explore whether chronic elevation of AChE-R affects behavioral and physiological functions, we employ FVB/N transgenic (Tg) mice over-expressing human AChE-R. The present report correlates behavioral and physiological functions with brain distribution of AChE-R and of cFOS, an immediate early gene that is responsive to stress. Tg AChE-R mice appeared healthy but their body weight was lower compared to FVB/N mice. Low body weight correlated with appearance of AChE-R-filled neurons in the lateral hypothalamic area and with increase in c-FOS positive cells in lateral and ventromedial hypothalamus. Compared to FVB/N mice, Tg-AChE-R mice were significantly impaired in learning in a serial choice maze. This was correlated with appearance of AChE-R-filled neurons and c-FOS positive cells in the hippocampal dentate gyrus. Additional mice, were implanted with transmitters and their motor activity recorded in the home-cage. Increased motor activity in Tg-AChE-R mice correlated with appearance of AChE-R-filled neurons and c-FOS positive cells in striatum, parietal and retrosplenial cortex. We propose that chronic elevation of AChE-R alters regulation of neuronal excitability in several brain regions that, in turn, may contribute to the alterations in behavioral and physiological functions under conditions of chronic stress.

STUDY ON THE MECHANISM OF BLOCKADE OF ACETYLCHOLINE RELEASE BY SNAKE PRESYNAPTIC PLA2 NEUROTOXINS ON NERVE TERMINALS

O. Rossetto, M. Rigoni, P. Caccin, C. Montecucco

Department of Biomedical Sciences, University of Padova, Italy

Several animal venoms contain toxins with phospholipase A2 (PLA2) activity. These enzymes hydrolyse the sn-2 ester bond of 1,2-diacyl-3-sn-phosphoglycerides producing fatty acids and lysophospholipids. Some snake venoms contains presynaptic PLA2 neurotoxins that cause a persistent blockade of neurotransmitter release from nerve terminals. Three subsequent phases can be distinguished at the neuromuscular junction (NMJ) poisoned by PLA2 neurotoxins: a short initial phase with either decreased or unchanged ACh release, is followed by a longer phase of facilitation of ACh release, which then fades into the third phase of complete and irreversible inhibition of neurotransmission. Electron microscopy studies of poisoned NMJ revealed appearance of many clathrin-coated W-shaped plasma membrane invaginations, indicating a blockage of endocytosis. Therefore PLA2 neurotoxins both promote fusion of small synaptic vesicles (SSVs) with the presynaptic membrane and inhibit their retrieval, thus causing release of ACh, depletion of vesicles and enlargement of nerve terminals. On the basis of these data we are investigating whether PLA2 neurotoxins block nerve terminals entering the lumen of synaptic vesicles and hydrolyse phospholipids of the inner leaflet of the membrane. The suggested entry of PLA2 neurotoxins inside SSVs would account for the finding that electrical activity of the nerve terminals actively promotes intoxication. In order to test our hypothesis we are studying the intracellular localization of two snake PLA2 neurotoxins (beta-bungarotoxin and taipoxin) on mouse phrenic nerve-hemidiaphragm NMJ intoxicated with these toxins. If this working hypothesis proves true, the presynaptic PLA2 neurotoxins could be employed as tools to investigate specific aspects of acetylcholine release and of vesicles fusion and recycling.

THE ROLE OF READTHROUGH ACETYLCHOLINESTERASE IN THE PATHOPHYSIOLOGY OF MYASTHENIA GRAVIS

T. Evron¹, Y. Hamra², N. Boneva², S. Seidman¹, T. Brenner², H. Soreq¹

¹*Department of Biological Chemistry, The Institute of Life Sciences, The Hebrew University of Jerusalem, ²Department of Neurology, Hadassah University Hospital and Hebrew University Hadassah Medical School, Jerusalem, Israel*

Alternative splicing induces, under cholinergic imbalances, overproduction of the rare "readthrough" acetylcholinesterase variant, AChE-R. We explored the pathophysiological relevance of this phenomenon in patients with myasthenia gravis (MG) and rats with experimental autoimmune MG (EAMG), both neuromuscular junction diseases with depleted acetylcholine receptors. In MG and EAMG, we detected serum AChE-R accumulation. In EAMG, we alleviated electromyographic abnormalities by nanomolar doses of EN101, an antisense oligonucleotide that selectively lowers AChE-R in blood and muscle, yet leaves unaffected the synaptic variant, AChE-S. While animals treated with placebo or conventional anticholinesterases continued to deteriorate, 4 weeks daily oral EN101 administration improved survival, neuromuscular strength and clinical status in moribund EAMG rats. The efficacy of targeting only one AChE splicing variant highlights potential advantages of mRNA-targeted therapeutics for chronic cholinergic imbalances.

"READTHROUGH" ACETYLCHOLINESTERASE FORMS NEURONAL COMPLEXES WITH PKC BETA II AND ITS WD CARRIER RACK1

E.H. Sklan, K.R. Birikh, S. Shoham, H. Soreq

Department of Biological Chemistry, The Life Sciences Institute, The Hebrew University of Jerusalem, Israel

Expression of neuronal "readthrough" acetylcholinesterase (AChE-R) is robustly increased under psychological stress, when it serves to hydrolyse the stress-elevated acetylcholine. However, the AChE-R protein also accumulates intracellularly.

Therefore, we used a yeast two hybrid screen to search for intracellular protein partners of ARP1 (AChE readthrough peptide 1), the C-terminal AChE-R domain that does not participate in acetylcholine hydrolysis. Here, we report that AChE-R, through ARP1, interacts intracellularly with RACK1, the WD domain PKC beta II carrier protein. Ex-vivo, triple ARP1/RACK1/PKC beta II complexes were found in transfected COS cells and AChE-R/RACK1/PKC beta II complexes in native pheochromocytoma cells. In vivo, physiological stress induced parallel increases in AChE-R, RACK1 and PKC beta II in parietal cortex and hippocampal CA1 neurons. Moreover, the stress-protected AChE-R overexpressing transgenic mice display elevated levels of RACK1 and PKC beta II, accompanied by RACK1 translocation to the perikaryal periphery and the appearance of dense co-labeled AChE-R/PKC beta II punctated neuronal clusters in several stress-responding brain regions. These findings present a non-catalytic intracellular capacity for AChE-R which may trigger PKC beta II-dependent signalling processes that augment physiological responses to diverse external stimuli.

CHRONIC ACETYLCHOLINESTERASE OVEREXPRESSION INDUCES MULTILEVELED ABERRATIONS IN NEUROMUSCULAR PHYSIOLOGY

N. Farchi, H. Soreq, B. Hochner

Departments of Neurobiology and Biological Chemistry, The Life Sciences Institute, The Hebrew University of Jerusalem, Israel

Overexpression of acetylcholinesterase (AChE) is a notable consequence of exposure to anticholinesterase drugs and/or poisons. However, the physiological consequences of such chronic overexpression with regards to neuromuscular function were not yet carefully analyzed. Here, we report detailed dissection of the different components of neuromuscular transmission in transgenic mice overexpressing the neuronal "synaptic" variant of AChE, which were previously reported to develop altered muscle morphology (Andres et al., 1997). Transgenic diaphragms presented pronounced fatigue, which reflected both neurotransmission fading and muscle mechanical aberrations. Under tetanic stimulation protocol, transgenic (TG) muscles fatigued to a larger extent than wild type (WT) muscles, either when stimulated directly (40% vs. 25% decay from initial force, within 10 contractions of 10 s intertrain interval, respectively) or via the phrenic nerve (68% vs. 49%, respectively), probably due to an impaired recovery process. AChE overexpression further affected synaptic transmission with significantly higher quantal content (0.095 in TG vs. 0.068 in WT, $P < 0.05$, at 0.2 mM Ca^{2+} and 2.4 mM Mg^{2+}). Furthermore, adjusted quantal size was exposed by treatment with physostigmine (10 μM) revealing higher amplitude (2.08 mV in TG vs. 1.54 mV in WT, $P < 0.01$) and half decay time (2.87 ms in TG vs. 2.29 ms in WT, $P < 0.02$) in the TG neuromuscular junction. Our observations predict multileveled neuromuscular malfunctioning under disrupted cholinergic homeostasis, which is relevant for chronic anticholinesterase exposure.

EXPRESSION OF THE CHOLINERGIC GENE LOCUS IN THE TRACHEAL EPITHELIUM OF THE RAT

U. Pfeil, L. Eberling, K.S. Lips, R.V. Haberberger, W. Kummer

Institute for Anatomy and Cell Biology, Justus-Liebig University, Giessen, Germany

Cholineacetyltransferase (ChAT) catalyzes the biosynthesis of acetylcholine (ACh) from acetyl-CoA and choline in the axoplasm. The vesicular acetylcholine transporter (VACHT) is responsible for the translocation of ACh into the interior of synaptic vesicles. The gene coding for ChAT contains the entire intronless sequence for VACHT within its first intron. Northern blot analyses have demonstrated mRNAs of different sizes in the central and peripheral nervous system of the rat. A ChAT mRNA (cChAT) of a single size (about 4 kb) was detected in brain and spinal cord. In peripheral tissues mRNAs of different sizes and a peripheral ChAT (pChAT) which seems to be a splice variant of cChAT could be detected. The occurrence of ChAT in non-neuronal tissue is known, but still unknown is the molecular identity of ChAT in the respiratory epithelium. Thus, we amplified the whole coding region of the rat tracheal epithelial ChAT mRNA. By RT-PCR a 2071 bp fragment could be detected, which shares a 99% sequence identity with cChAT. The expression of VACHT could be demonstrated for the first time in rat tracheal epithelium by RT-PCR. Amplification of the whole coding region revealed a sequence identity of about 99 % to the VACHT known from cholinergic neurons. In the present study we demonstrate the simultaneous expression of ChAT and VACHT in rat tracheal epithelium. The obtained sequencing data strongly suggest that the ChAT and VACHT proteins of rat tracheal epithelium are identical to that known from central cholinergic neurons. (SFB 547, project C2)

MUSCARINIC RECEPTORS AND TRP-CHANNELS IN PRIMARY SENSORY NEURONS OF THE RAT

R. Haberberger¹, S. Wiegand¹, M. Kress²

¹*Institute for Anatomy and Cell Biology, Justus-Liebig-University Giessen,*

²*Institute for Physiology, University Erlangen-Nürnberg, Germany*

In sensory neurones acetylcholine interacts with G-protein coupled muscarinic receptors (MR), thereby activating a variety of second messenger systems (IP₃, DAG, PLC, PKC). TRP-channels are a family of Ca²⁺-permeable cation channels that are activated by depletion of intracellular Ca²⁺-stores or subsequent to the stimulation of PLC isoforms. In *in vitro* expression systems, MR are coupled via Gq/11 proteins to transient receptor potential (TRP) channels. Stimulation is followed by an increase in [Ca²⁺]_i. Similarly, MR activation leads to an increase in [Ca²⁺]_i in sensory dorsal root ganglion (DRG) neurons. Therefore, we investigated the presence and localisation of TRP-channels in DRG neurons at transcriptional (RT-PCR), translational (immunohistochemistry) and functional (Ca²⁺-imaging) level. Total RNA of lumbar DRG contained mRNA for five out of seven channel subtypes. Proteins for the TRP1-, 3- and 6-channel could be demonstrated in subpopulations of sensory neurons. Perikarya that expressed the marker of presumably nociceptive neurons, the vanilloid receptor-1, were also immunoreactive for the TRP1-, 3- and 6-channels. Activation of Gq/11-coupled MR using muscarine was followed by an increase in [Ca²⁺]_i. The rise in [Ca²⁺]_i was abolished in presence of Nickel/Cadmium or after depletion of intracellular Ca²⁺-stores by application of thapsigargin. This further suggests the involvement of TRP-channels in the MR-mediated Ca²⁺-signalling. (SFB 547, Teilprojekt C2; SFB 353, Teilprojekt A10)

DIVERSE MOLECULAR MECHANISMS UNDERLYING CONGENITAL MYASTHENIC SYNDROMES

R.G. Webster¹, R. Croxson¹, S. Brownlow¹, M. Brydson¹, S. Haslam¹, C. Young², C. Slater², J. Newsom-Davis¹, A. Vincent¹, D. Beeson¹

¹*Neuroscience Group, Weatherall Institute of Molecular Medicine, Oxford,*

²*Department of Neurobiology, University of Newcastle, UK*

Mutations of muscle acetylcholine receptors cause defective neuromuscular transmission through a variety of molecular mechanisms. AChR deficiency is the most common of the congenital myasthenic syndromes (CMS). However, it is not always clear how mutations within the AChR epsilon-subunit coding regions affect mRNA and protein levels at the endplate. We found different homozygous mutations, located in the M3-M4 cytoplasmic loop, in four patients with typical AChR deficiency. Surface expression in HEK293 cells showed that each is a null mutation. Surprisingly, *in situ* hybridisation in biopsies showed normal expression of epsilon-subunit mRNA. Thus the mutant epsilon-subunit mRNA transcripts are neither up-regulated nor preferentially degraded. Moreover, there was no compensatory increase in gamma-subunit mRNA suggesting that the normal low level of the gamma-subunit in human muscle is sufficient for survival of patients with epsilon-subunit null alleles.

Other CMS are due to abnormal ion channel function. In one case, abnormal fetal development had resulted in multiple joint contractures (arthrogryposis) of the fingers at birth, as well as CMS presenting in infancy. Mutational screening revealed heteroallelic mutation within the AChR delta-subunit, d756ins2 and dE59K. d756ins2 is a null mutation, but both adult and fetal AChRs containing dE59K show abnormally short burst lengths, predicting a "fast channel" phenotype. Thus dE59K causes dysfunction of fetal as well as adult AChRs explaining the presence of joint contractures which result from reduced fetal movement. This is the first description of the association of AChR gene mutations and arthrogryposis, but any mutation that disrupts fetal AChR function could underlie additional cases.

ACETYLCHOLINESTERASE KNOCKOUT MICE HAVE INCREASED SENSITIVITY TO SCOPOLAMINE AND ATROPINE

A. Hrabovska^{1,2}, O. Lockridge¹, E. Duysen¹

¹*Eppley Institute, University of Nebraska Medical Center, Omaha, NE, USA,*

²*Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic*

It is generally accepted that continued stimulation of cells with agonists results in a state of desensitization or downregulation. The purpose of this work was to test the hypothesis that AChE ^{-/-} mice have reduced levels of functional muscarinic receptors. The toxicity of the antimuscarinic drugs, scopolamine and atropine, was tested in acetylcholinesterase wild-type (AChE ^{+/+}), heterozygous (AChE ^{+/-}) and nullizygous mice (AChE ^{-/-}). Mice were injected i.p. with scopolamine or atropine dissolved in saline. In the case of a lethal dose, mice died within 2-15 minutes. The LD₅₀ for scopolamine was 100 mg/kg in AChE ^{+/+}, 180 mg/kg in AChE ^{+/-}, and 35 mg/kg in AChE ^{-/-} mice. The LD₅₀ for atropine was 250 mg/kg in AChE ^{+/+}, 215 mg/kg in AChE ^{+/-}, and 96 mg/kg in AChE ^{-/-} mice. The higher sensitivity of AChE ^{-/-} mice to muscarinic antagonists is consistent with the interpretation that these mice have fewer muscarinic receptors. These results correlate with data obtained in our laboratory (Bin Li et al.) demonstrating that AChE ^{-/-} mice are less sensitive to the muscarinic agonists, oxotremorine and pilocarpine. Taken together, these results support the conclusion that AChE ^{-/-} mice have adapted to excess acetylcholine by downregulating muscarinic receptors.

Supported by U.S. Army Medical Research & Materiel Command Grant DAMD17-01-2-0036

DOWNREGULATION OF MUSCARINIC RECEPTORS IN MICE DEFICIENT IN ACETYLCHOLINESTERASE

B. Li, E.G. Duysen, O. Lockridge

Eppley Institute, University of Nebraska Medical Center, Omaha, NE, USA.

This study examined how acetylcholinesterase (AChE) knockout mice have adapted to the absence of AChE. AChE hydrolyzes acetylcholine to terminate cholinergic neurotransmission. Overstimulation of cholinergic receptors by excess acetylcholine is lethal. However, AChE^{-/-} mice live to adulthood. The hypothesis was tested that adaptation occurred through downregulation of cholinergic receptors. Muscarinic receptors were investigated by treating mice with muscarinic receptor agonists, pilocarpine and oxotremorine (OXO). In response to 200 mg/kg pilocarpine i.p., 10/10 AChE^{+/+}, 5/10 AChE^{+/-}, and 0/6 AChE^{-/-} mice had seizures. Results indicated that AChE^{-/-} mice have reduced numbers of functional muscarinic receptors. A second group of mice was treated with 1 mg/kg OXO s.c. OXO specifically stimulates muscarinic receptors inducing tremor, hypothermia, and salivation. 6/6 AChE^{+/+}, 6/6 AChE^{+/-}, and 0/6 AChE^{-/-} mice had severe tremor, a drop in surface body temperature of 12

TARGETING OF THE HUMAN VESICULAR ACETYLCHOLINE TRANSPORTER TO CHOLINERGIC SUBDIVISIONS IN TRANSGENIC MICE

B. Schuetz¹, E. Weihe², L.E. Eiden³

¹Laboratory on Molecular Neurobiology, Clinic for Psychiatry, University of Bonn, Bonn, Germany. ²Department of Molecular Neuroscience, Institute of Anatomy and Cell Biology, University of Marburg, Germany.

³Laboratory on Cellular and Molecular Regulation, National Institute of Mental Health, Bethesda, MD, USA

The vesicular acetylcholine transporter (VACHT) and choline acetyltransferase (ChAT) are encoded within a single regulatory unit, called the cholinergic gene locus (CGL). To identify regions in the CGL that are important for the cell type-specific expression of VACHT *in vivo*, we tested fragments of the human CGL for their ability to confer correct expression of human VACHT to mouse cholinergic neurons. In our previous work (Neuroscience 96 (2000):707-22) we identified an 8.7 kb fragment from the human CGL that restricted expression of the embedded VACHT to somatomotor neurons in transgenic mice. In the present study, we report the generation and analysis of two additional human CGL transgenes. The addition of 2.5 kb of downstream sequence to the existing transgene resulted in an extension of the expression of human VACHT to the cholinergic neurons of the medial habenular nucleus. The removal of 4.5 kb from the 5'-end of this construct completely abolished human VACHT expression in mice. Our data provide strong evidence for a mosaic model for CGL regulation in separate subdivisions of the mammalian cholinergic nervous system.

THE DIURNAL ACTIVITY OF ACETYLCHOLINESTERASE INHIBITORS

B.M. Davies

General Practice, Syosset, NY, USA

Objective: Acetylcholinesterase inhibitors (AChEI), particularly donepezil, can induce sleep disorders in Alzheimer's disease patients. It is believed that acetylcholinesterase (AChE) displays diurnal variation, and that this circadian phenomenon may be linked to AChEI-induced sleep disturbances. We examined the potential effects of AChEI on the diurnal activity of AChE. **Methods:** A literature search was conducted to identify studies investigating the sleep-activity cycle of patients taking AChEI. **Results:** Published evidence suggests that AChE activity follows a circadian pattern. Drugs such as donepezil, which exhibit highly potent AChEI activity, seem to interrupt this natural diurnal activity. As donepezil has a long half-life (average, 70 hours), it remains in the body for long periods of time. Thus, even if given in the morning, donepezil concentrations can still remain high enough to create daytime acetylcholine levels at night, interrupting sleep. Donepezil may override the circadian rhythm of the cholinergic system, disturbing the sleep-activity cycle and resulting in high incidences of insomnia and other sleep-related events, and raising hypnotic medication use. Galantamine (Reminyl(R)) has a half-life of 6-8 hours, is a less potent AChEI than donepezil, and allosterically modulates nicotinic acetylcholine receptors. Unlike donepezil, galantamine appears not to perturb the diurnal activity of AChE. This could explain the lower incidence of sleep disturbances and concomitant hypnotic use with galantamine. **Conclusion:** While donepezil may override the natural diurnal activity of AChE resulting in increased sleep disturbance, galantamine appears not to perturb this activity, giving a lower incidence of insomnia and sleep-related disorders.

THE MUSCARINIC M1 RECEPTOR AS A THERAPEUTIC TARGET FOR COGNITIVE DEFICITS: PRECLINICAL PHARMACOLOGY AND KNOCKOUT MOUSE STUDIES

C.C. Felder¹, K.S. Gannon², F.P. Bymaster², A. Porter², D.L. McKinzie², J. Wess⁴, N.M. Nathanson³

¹Eli Lilly and Co., Neuroscience Division, Windlesham, Surrey, UK.

²Eli Lilly and Co., Neuroscience Division, Indianapolis, IN. ³University of Washington Medical Center, Department of Pharmacology, Seattle, WA.

⁴NIDDK, Laboratory of Bioorganic Chemistry, Bethesda, MD, USA

Muscarinic M1 receptors are localised to brain regions involved in learning and memory and have been implicated in the regulation of cognitive processes at the behavioural and molecular level. However, the specific muscarinic receptor subtypes involved in cognitive processing have not been definitively identified. With the availability of muscarinic receptor knockout mice, the effects of various receptor subtypes on learning and memory can be examined. In the present studies, spatial learning and memory as well as open field activity were assessed in M1 knockout (KO) mice and age-matched wild type (WT) controls. Significant deficits were found for M1-KO mice compared to controls in task completion time, reference memory and total errors. M1-KO mice had a significantly greater rate of ambulation on Days 1, 3 and 14. Results indicate that M1-KO mice exhibit impaired learning and memory in a spatial discrimination task and that this effect was independent of differences in activity level which were short-lived with extended monitoring. Within the hippocampus, the M1 receptor, but not the functionally similar M3 receptor, stimulated G protein activation and calcium mobilizing signaling pathways. Broader studies with mice bearing a genetic deletion of each of the 5 muscarinic receptor subtypes suggests that muscarinic receptor-mediated activities, such as tremor, salivation, hypothermia, heart rate, and smooth muscle function are regulated predominantly through the M2 and M3 receptors. Taken together, these studies indicate that the M1 receptor is a likely target for the development of therapeutics for the treatment of cognitive deficits.

RESCUE OF THE ACETYLCHOLINESTERASE KNOCKOUT MOUSE BY FEEDING A LIQUID DIET; PHENOTYPE OF THE ADULT ACETYLCHOLINESTERASE DEFICIENT MOUSE

E.G. Duysen¹, J.A. Stribley², D. Fry¹, S. Hinrichs², O. Lockridge¹

¹University of Nebraska Medical Center, Eppley Institute, ²University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, NE, USA

Acetylcholinesterase (AChE, EC3.1.1.7) functions in nerve impulse transmission, and possibly as a cell adhesion factor during neurite outgrowth. These functions predicted that a mouse with zero AChE activity would be unable to live. It was a surprise to find that AChE ^{-/-} mice were born alive and survived an average of 14 days. The emaciated appearance of AChE ^{-/-} mice suggested an inability to obtain sufficient nutrition and experiments were undertaken to increase caloric intake. Pregnant and lactating dams (+/-) were fed 11% high fat chow supplemented with liquid Ensure. AChE ^{-/-} pups were weaned early, on day 15, and fed liquid Ensure. Although nullizygous animals showed slow but steady weight gain with survival over 1 year (average 100 days), they remained small at all ages compared to littermates. They demonstrated delays in temperature regulation (day 22 vs 15), eye opening (day 13 vs 12), righting reflex (day 18 vs 12), descent of testes (week 7-8 vs 4), and estrous (week 9 vs 6-7). Significant physical findings in adult AChE ^{-/-} mice included body tremors, abnormal gait and posture, absent grip strength, inability to eat solid food, pinpoint pupils, decreased pain response, vocalization, and early death caused by seizures or gastrointestinal tract ileus. Behavioral deficits included urination and defecation in the nest, lack of aggression, reduced pain perception, and sexual dysfunction. These findings support the classical role for AChE in nerve impulse conduction and further suggest that AChE is essential for timely physical development and higher brain function.

BRAIN PENETRATION AND BEHAVIOURAL PROPERTIES OF A POTENT ALPHA 7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST IN THE RAT

N.M. Moore¹, D.L. McKinzie², S.N. Mitchell¹, M. Keenan¹, G. Wishart¹, T.K. Murray¹, B. Tree¹, S. Iyengar², J. Hart², D. Shaw², R. Simmons², A. Kalra², C. Miles¹, J.R. Boot¹, S.R. Baker¹, E. Sher¹, M.D. Tricklebank¹, M.J. O'Neill¹

¹Eli Lilly and Company, Erl Wood Manor, Windlesham, Surrey, UK,

²Eli Lilly and Company Ltd., Lilly Corporate Centre, Indianapolis, IN, USA

The homomeric alpha-7 nicotinic receptor is the second most abundant nicotinic receptor in the brain and has been implicated in a number of psychiatric and neurological disorders. We have evaluated PSAB-OPF ((R)-(+)-5'-phenylspiro[1-azabicyclo[2.2.2]octane-3,3'(3'H)-furo[2,3-b]pyridine], Phillips et al., Astra Arcus USA, patent WO99/03859 a potent alpha-7 agonist in a battery of behavioural assays in the rat. Initial studies confirmed that after systemic administration (5 mg/kg s.c.) the C_{max} in rat brain was 5.8 microM with a T_{1/2} of 1.3 hr. We then went on to evaluate the effects in the rat of PSAB-OPF in locomotor activity, pre-pulse inhibition and on performance in the radial and Morris mazes. In addition the compound was examined against hyperalgesia induced by formalin and carrageenan and in 6-hydroxydopamine lesioned rats. Results indicated that PSAB-OPF decreased spontaneous locomotor activity 20-40 min after injection (20 % decrease at 10 mg/kg), but failed to alter stimulant-induced activity, pre-pulse inhibition or cognitive performance in either the Morris water maze or 8-arm radial maze. The compound was also inactive in hyperalgesia models and had no functional or neuroprotective actions in the 6-OHDA model. PSAB-OPF was thus inactive in a wide range of behavioural assays. It is not clear whether this reflects a relatively unimportant role for the alpha-7 receptor, insufficient receptor exposure to the compound and/or rapid receptor desensitisation. The 5-HT₃ agonist cross-reactivity of this molecule also makes it difficult to make concrete conclusions on the role of alpha-7 receptors in these in vivo tests.

ROLE OF MUSCARINIC RECEPTORS IN THE ACTIVATION OF THE SUBICULO-ACCUMBENS PROJECTION

S.N. Mitchell, S. Moss, A. Sharott

Eli Lilly and Company Ltd., Windlesham, Surrey, UK

The nucleus accumbens receives limbic inputs from a number of brain regions, including the ventral subiculum. Activation of this projection, following microinjection of N-ethyl-D-aspartate (NMDA) or carbachol increases locomotor activity. Using in vivo microdialysis, ventral subiculum application of NMDA increases levels of dopamine in the nucleus accumbens. Experiments were conducted to ascertain, in the nucleus accumbens, the neurochemical consequences of carbachol administration using microdialysis, and to explore the cholinergic receptor subtype(s) involved in any evoked response. In anaesthetised rats, ventral subiculum administration of carbachol increased dopamine levels in the nucleus accumbens. Administration of nicotine or the alpha-7 nicotinic acetylcholine receptor agonist, AR-R17779 failed to evoke a response. An involvement of muscarinic receptors was suggested from the significant reduction in response to carbachol following co-administration with atropine. Sensitivity of the subiculo-accumbens projection to muscarinic cholinergic receptor stimulation was confirmed by a significant increase in nucleus accumbens dopamine following ventral subiculum administration of the broad-spectrum muscarinic agonist oxotremorine M. In further studies utilising subtype selective agonists, xanomeline (M1 and M4 preferring agonist) failed to increase dopamine. However, ([5R-[exo]-6-[butylthio]-1,2,5-thiadiazol-3-yl]-1-azabicyclo[3.2.1]octane (BuTAC - M2 and M4 partial agonist, and M1, M3 and M5 antagonist) evoked a significant response. These data show that the subiculo-accumbens projection is sensitive to muscarinic receptor stimulation, and suggests an involvement of the M2 receptor subtype.

FINE-TUNING MODULATION OF NEURONAL MUSCARINIC M1 (FACILITATORY) AND M2 (INHIBITORY) RECEPTORS ACTIVATION BY ADENOSINE AT THE RAT NEUROMUSCULAR JUNCTION

L. Oliveira, M.A. Timoteo, P. Correia-de-Sa

Laboratorio de Farmacologia, UMIB, Instituto de Ciencias Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Portugal

The crosstalk between adenosine and muscarinic autoreceptors regulating evoked [3H]-acetylcholine ([3H]-ACh) release was investigated on rat phrenic nerve-hemidiaphragms. Motor nerve terminals possess facilitatory M1 and inhibitory M2 autoreceptors. Dicyclomine (3 nM-10 microM) caused a biphasic (inhibitory/facilitatory) effect, indicating that M1-facilitation prevails during 5 Hz-trains. Co-activation of M2 receptors was partially attenuated, since pirenzepine (1 nM, an M1-antagonist) significantly enhanced inhibition by oxotremorine (10 microM). CGS 21680C (2 nM), an A2A-adenosine receptor agonist, (1) potentiated oxotremorine (10 microM) inhibition, and (2) shifted McN-A-343 (3 microM)-facilitation into a small inhibitory effect. Conversely, the A1-receptor agonist, R-PIA (100 nM), reduced the inhibitory effect of oxotremorine (10 microM), without changing facilitation by McN-A-343 (3 microM). Synergism between A2A- and M2-receptors is regulated by a reciprocal interaction with M1 receptors that can be prevented by pirenzepine (1 nM). During 50 Hz-bursts, facilitation (M1) of [3H]-ACh release by McN-A-343 (3 microM) disappeared, while M2 inhibition became predominant. This muscarinic shift results from the interplay with A2A-receptors, because it was prevented by the A2A antagonist, ZM 241385 (10 nM). Thus, when muscarinic M1 facilitation is fully operative, inhibition of ACh release is mediated by adenosine A1 receptors. During high frequency bursts, tonic activation of A2A receptors promotes M2-autoinhibition by braking M1 receptor counteraction.

Work supported by FCT.

IDENTIFICATION OF SIGNALING PROTEINS DOWNSTREAM OF THE TYROSINE KINASE MUSK IN CLUSTERING OF ACETYLCHOLINE RECEPTORS

R. Willmann, P. Mittaud, C. Fuhrer

University of Zurich, Switzerland

During development of the neuromuscular junction, nerve-released agrin causes clustering of acetylcholine receptors (AChRs) in the muscle membrane. The muscle specific kinase (MuSK) is part of the agrin receptor complex and undergoes agrin-induced dimerization and autophosphorylation, thereby starting a signaling pathway that eventually drives AChR phosphorylation and clustering. We observed previously that treatment of C2C12 myotubes with the kinase inhibitor staurosporine prevents aggregation of AChRs but leaves MuSK active, implying the activity of other kinases downstream of MuSK. To analyse possible interaction partners and/or substrates of MuSK, we immunoprecipitated MuSK and identified associated phosphorylated proteins by phosphotyrosine immunoblotting. One major band running at about 60 kDa was specifically coprecipitated by MuSK and phosphorylated on tyrosine in response to agrin treatment. As Src-family kinases bind to the AChR and are activated by agrin, we investigated the possibility that these kinases associate with MuSK and are phosphorylated due to agrin. In myotubes derived from mice lacking both Src and Fyn, no change in the phosphorylation extent of the MuSK-associated protein was observed. Staurosporine and the specific Src-family kinase inhibitors PPI and CGP77675 did not affect the phosphorylation degree of the protein. Instead, it was reduced by treatment with herbimycin, in parallel with the reduction of MuSK phosphorylation. Finally, in myotubes lacking rapsyn, phosphorylation of the MuSK-associated protein was still observed. Thus, so far, the identification of the MuSK-associated protein as a member of the Src-family could not be confirmed, and we presently consider other muscle proteins of 60 kDa.

CHEMICAL MODIFICATION OF RECOMBINANT HUMAN ACETYLCHOLINESTERASE BY POLYETHYLENE GLYCOL GENERATES AN ENZYME WITH EXCEPTIONAL CIRCULATORY LONGEVITY

O. Cohen, C. Kronman, T. Chitlaru, S. Lazar, N. Seliger, D. Kaplan, A. Ordentlich, B. Velan, A. Shafferman

Department of Biochemistry and Molecular Genetics, Israel Institute for Biological Research, Ness-Ziona, Israel

One of the major obstacles to the fulfillment of the therapeutic potential of recombinant human acetylcholinesterase (rHuAChE) as a bioscavenger of organophosphates is its short circulatory residence. Post-translation-related factors such as sialylation level and subunit assembly were recently shown to determine the circulatory fate of AChE and demonstrated the ability to generate recombinant AChE with improved pharmacokinetic traits. Here we show that the pharmacokinetic performance of rHuAChE can be increased significantly by the controlled attachment of polyethylene glycol (PEG) side-chains to lysine residues. The increase in mean residence time (MRT) of the PEG-modified monomeric enzyme is linearly dependent, in the tested range, on the number of attached PEG molecules as well as on their size. It appears that even low level PEG-conjugation can overcome the deleterious effect of sub-optimal post-translation modifications such as under-sialylation. Attachment of as many as four PEG molecule to monomeric rHuAChE had minimal effect if any on either the catalytic activity or on the reactivity of the modified enzymes towards active center inhibitors such as edrophonium and diisopropylfluorophosphate (DFP) or to peripheral site ligands such as propidium, BW284C51 and even towards the bulky snake-venom toxin - fasciculins. At the highest tested ratio of attached PEG-20,000 to rHuAChE (4:1), an MRT of over 2100 minutes was attained (compared to MRT of 42 minutes for the non-modified enzyme), a value unmatched by any other known form of recombinant or native plasma derived AChE reported to date. This provides an important step toward the generation of a pharmaceutically efficient recombinant AChE-based bioscavenger for prophylactic treatment of organophosphate poisoning.

This work was supported by the U.S. Army Research and Development Command, Contract DAMD17-00-C-0021 (to A.S.).

DRAMATIC DEPLETION OF CELL SURFACE ACETYLCHOLINE MUSCARINIC RECEPTORS m2R DUE TO LIMITED DELIVERY FROM INTRACYTOPLASMIC STORES IN NEURONS OF ACETYLCHOLINESTERASE (ACHE) DEFICIENT MICE

V. Bernard¹, C. Brana², I. Liste¹, O. Lockridge³, B. Bloch¹

¹CNRS UMR 5541, Laboratoire d'Histologie-Embryologie, Université Victor Segalen-Bordeaux 2, ²Laboratoire d'Epileptologie Experimentale et Clinique, Université Victor Segalen-Bordeaux 2, France. ³Eppley Institute, University of Nebraska, Omaha, USA

The abundance of G protein-coupled receptors (GPCR) located at the neuronal membrane depend on complex intraneuronal trafficking involving delivery of GPCRs from the cytoplasm to the membrane. We have studied here the effect of the chronic AChE deficiency in neurons of AChE gene KO mice on the subcellular distribution of the m2R, using immunohistochemistry at light and electron microscopic levels. 1) In AChE^{+/+} mice in vivo, m2R is abundant at the plasma membrane in striatum, hippocampus and cortex. 2) In AChE^{-/-} mice, m2R is almost absent at the membrane but is abundant in endoplasmic reticulum and Golgi complex. 3) Dynamic studies show that the balance between membrane and cytoplasmic m2R depends on the cholinergic influence: a) In AChE^{-/-} mice, the blockade of muscarinic receptors restores m2R at the membrane. b) In AChE^{-/-} mice in vitro (organotypic culture), when acetylcholine is produced by interneurons (striatum), m2R is located in the cytoplasm as in vivo. The supplementation of AChE^{-/-} neurons with AChE provokes a translocation of m2R from the cytoplasm to the membrane. c) In vitro, when AChE^{-/-} hippocampus is disconnected from its cholinergic afference, m2R is located at the membrane. When AChE^{-/-} hippocampus is co-cultured with AChE^{-/-} septum, its cholinergic afference, m2R keeps the cytoplasmic distribution seen in hippocampus in vivo. Our data suggest that the neurochemical environment may contribute to the control of abundance and availability of cell membrane GPCR, and consequently to the control of neuronal sensitivity to neurotransmitters, by regulating their delivery from intracytoplasmic stores to the membrane.

HUPERZINE A AND DONEPEZIL ATTENUATE STAUROSPORINE-INDUCED APOPTOSIS IN RAT CORTICAL NEURONS VIA BCL-2 AND BAX REGULATION AND INHIBITION ON CASPASE-3

H.Y. Zhang, X.C. Tang

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes For Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Staurosporine treatment results in apoptotic cell death and DNA fragmentation. bcl-2, bax and Caspase-3 are known to regulate the apoptotic cell death. This study sought to examine effects of huperzine A and donepezil on staurosporine-induced neuronal apoptosis and potential mechanisms in primary cultured rat cortical neurons. Treated with 0.5 micromolar staurosporine for 24 hours results in significant decrease in cell viability, alteration of neuronal morphology and DNA fragmentation. Pretreatment of the cells with huperzine A and donepezil for 2 hours prior to staurosporine exposure markedly elevated the cell survival at 0.1-10 micromolar concentration and reduced staurosporine-induced nuclei fragmentation at 1 micromolar concentration. 1 micromolar huperzine A and donepezil pretreatment also reduced the upregulation of pro-apoptotic gene bax, the downregulation of anti-apoptotic gene bcl-2 as well as activation of Caspase-3. Thus our results provide the first evidence that huperzine A and donepezil protect neurons against staurosporine-induced apoptosis via the upregulation of bcl-2 and downregulation of bax and inhibition on Caspase-3 activity.

**INSECT GROWTH REGULATORS INHIBIT
ACETYLCHOLINESTERASE ACTIVITY IN B-BIOTYPE
BEMISIA TABACI IN AUSTRALIA**

E.L.A. Cottage¹, R.V. Gunning²

¹Dept. Agronomy & Soil Sc., University of New England, Armidale, NSW, Australia, ²NSW Agriculture, Tamworth Centre for Crop Improvement, Tamworth, NSW, Australia

B-biotype *Bemisia tabaci* (Sternorrhyncha: Aleyrodidae) was first detected in Australia in October 1994 and poses a threat to many plant industries. This insect is a severe pest worldwide and is resistant to most conventional insecticides. Insect growth regulators such as buprofezin and novaluron, are being investigated for use against B-biotype *B. tabaci* in Australia, although resistance has already been detected. In addition to disrupting the nymphal moulting process, we have found that buprofezin and novaluron are inhibitors of acetylcholinesterase in insect growth regulator susceptible Australian B-biotype *B. tabaci*. This is a new mode of action for insect growth regulators. This inhibition did not occur in the buprofezin resistant strain and our results indicate that insect growth regulator resistant B-biotype *B. tabaci* have evolved an insensitive form of acetylcholinesterase as a resistance mechanism.

**BIOCHEMICAL CHARACTERISATION OF MICE TRANSGENIC
FOR A MUTATION IN AMYLOID PRECURSOR PROTEIN (APP)
KNOWN TO CAUSE FAMILIAL ALZHEIMER'S DISEASE**

P.T. Francis¹, K.L. Matthews¹, K.E. Heslop¹, P.F. Chapman²

¹Centre for Neuroscience King's College London, UK,

²School of Biosciences, Cardiff University, UK

Alzheimer's disease (AD) is characterised by a deficit in markers of the cholinergic system (Francis et al, 1999). Tg2576 mice, which overexpress a transgene of human APP carrying the Swedish mutation (K670N, M671L), demonstrate age-dependant amyloid deposition, senile plaques and cognitive impairment (Hsiao et al., 1996). To investigate whether these mice also show cholinergic dysfunction, regional biochemical markers of this system were determined.

Brains from Tg2576 mice and non-transgenic (non-Tg) littermates of 3 ages (4, 8 and 13 months) were dissected to yield cortex, hippocampus and striatum and frozen (-70 °C) until assayed. Membranes were prepared as previously described and binding to receptors determined using a single ligand concentration (Minger et al, 2000).

Cortical muscarinic M1 and M2 binding was significantly reduced (by 70% and 60% respectively) in Tg2576 mice compared to non-Tg littermates at 13 months of age. There were no significant reductions in younger age groups, although a 25-30% decrease was seen in these receptors in 8 month old animals. No significant changes in binding to nicotinic receptor subtypes (alpha 4 beta 2 or alpha 7) were seen.

In conclusion, although the loss of muscarinic receptors in the oldest Tg2576 mice exceeds that seen in AD, it does coincide with cognitive impairment and Aβ deposition in these animals (Hsiao et al, 1996).

We thank The Wellcome Trust.

Francis P.T. et al. (1999) *J. Neurol. Neurosurg. Psychiatry* 66, 137-147.

Hsiao K. et al. (1996) *Science* 274, 99-102.

Minger S.L. et al. (2000) *Neurology* 55, 1460-1467.

**VESICULAR ACETYLCHOLINE TRANSPORTER TRAFFICKING
AND ADAPTOR PROTEINS**

M-H. Kim, L.B. Hersh

Department of Biochemistry, University of Kentucky, Lexington, KY, USA

In neuronal cells, the neurotransmitter acetylcholine is transported from the cytoplasm into synaptic vesicles by the vesicular acetylcholine transporter (VACHT). During biogenesis VACHT is trafficked into the synaptic vesicle. Adaptor proteins have been shown to play a major role in synaptic vesicle recycling. It has been proposed that the cytoplasmic tail of VACHT is involved in its trafficking to the small synaptic vesicle.

In this study we evaluated whether VACHT is sorted into synaptic vesicles through clathrin associated protein complexes. We constructed a GST-fusion VACHT cytoplasmic carboxyl tail and this was incubated with a rat brain extract. Complexes were captured on glutathione beads and analyzed by immunoblotting with Adaptor gamma to detect Adapter Protein-1 (AP-1) complexes, Adaptor alpha to detect AP-2 containing complexes, and AP-180 to detect AP-180 containing complexes.

Surprisingly, AP-1, AP-2, and AP-180 proteins were all detected indicating that they all bound to the VACHT cytoplasmic tail. We also tested mutants at the phosphorylation site and at the dileucine motif of VACHT to map the site of interaction with adaptor proteins. Mutant Leu485/486Ala showed no binding to AP-1, however binding to the AP-2 and AP-180 was retained. Mutants Ser480Ala and Ser480Glu retained binding to the APs but showed different binding intensities indicating that the relative amounts of these complexes changed.

On the basis of these results, we propose that VACHT trafficking involves association with clathrin associated protein complexes, and the cytoplasmic carboxyl tail of VACHT contains multiple trafficking signals.

**SYMPATHETIC SUPERIOR CERVICAL GANGLIA (S.C.G.) OF
CAT CHOLINERGIC RELAY OF
HYPOTHALAMIC-STIMULATED ORGAN-SPECIFIC VASCULAR
CHANGES-RELEVANCY TO NORMAL AND TO CLINICAL
DYSAUTONOMIC FUNCTION**

Baruch Blum, Jacob Israeli

Department of Physiology and Pharmacology, Tel Aviv University, Israel

Electrical stimulation of prefrontal lateral hypothalamus (LH) induces a moderate systemic BP rise. We examined this in cat model whether it is result of a general systemic or a localized vascular bed effect. Muscarinic and nicotinic mediation of sympathetic ganglia neurotransmission has been shown by use of ganglionic blockers ATMN & Hexamethonium. We obtained potentiation of the LH-induced BP rise by systemic ATMN or applied in vivo in situ to the desheathed s.c. ganglia thus showing the BP rise cholinergic muscarinic mediation at the ganglia, proposing this a blocking of a ganglionic attenuating mechanism. This cholinergic muscarinic mediation of sympathetic ganglia relay appeared to inherently allow specificity in target activation. Our study has confirmed that this BP rise is a result of discrete localized blood flow changes in just one or two individual organs: it is abolished by bilateral cutting of either one of s.c.g. exiting nerves the medial or the lateral branch. In compliance with the concept of segmental sympathetic innervation the respective s.c.g. exiting nerves were traced and identified in vivo, the medial to pharyngeal and to lower respiratory regions, the lateral branch to neck vasculature. Organ-specific blood flow neuro-regulation via segmental autonomic output from s.c.g. was thus demonstrated- as differentially innervating, neck vs pharyngeal and lower respiratory organs. While normal significance of this finding is implicit, we suggest possible bearing on dysautonomic swallowing into the trachea due to anomalous function of the innervation.

CATECHOLAMINE INDUCED CYTOTOXICITY AND ITS PROTECTION, TAURINE AND ANALOGUES: SOME MORE THOUGHTS

R.C. Gupta

Department of Chemistry, SASRD, Medziphema, India

Taurine, (2-Amino ethane sulfonic acid), a sulfur amino acid is present in the high concentration in mammalian tissues and involved in a diverse array of biological actions, osmoregulation, stabilization, bile salt synthesis and neuromodulation. It is also patented for epilepsy, hypertension. Taurine's participation in cellular antioxidant defense mechanisms has been recorded with scavenging of free radicals and their binding by reactive quinones, thus inhibiting free radicals formation. Taurine is also present in those tissues that contains high concentration of catecholamines which known to be cytotoxic and also to increase oxidative stress. Catecholamines are also known to undergo auto oxidations to generate free radical and cytotoxic quinones. It is observed that taurine directly inhibit L-dopa oxidation catalysed by metal like iron; thus this ability of taurine to inhibit metal stimulated catecholamine oxidation may have therapeutic implications, in diseases that are resulted with catecholamine induced oxidative stress and free radicals. As amino acid therapy has its own limitations taurine analogues are issues for other CNS actions, it will be thoughtful to utilize potential of taurine analogues in the therapy of catecholamine induced cytotoxicity.

THE EFFECT OF DIET RESTRICTION, SEPARATION STRESS AND TYROSINE ADMINISTRATION ON THE CHOLINERGIC SYSTEM IN MICE

S. Hao, Y. Avraham, S. Mendelson, E.M. Berry

Department of Human Nutrition and Metabolism, Hebrew University of Jerusalem, Hadassah Medical School, Jerusalem, Israel

Background: We have previously shown that weight loss leads to alterations in autonomic tone which may affect cognitive function. We have investigated the effect of tyrosine on hippocampal cholinergic activity in two animal weight loss models - Diet restriction (DR) and self-induced weight loss caused by Separation stress. **Methods:** Female Sabra mice were fed either 100% (control), or DR to 60% and 40% of daily nutritional requirements. Mice in the Separation group were housed in a cage fitted with six individual Plexiglas partitions of size 11x10x12 cm. They could smell each other and see their neighbours without physical contact except when transferred to regular cages for the 2-h feeding schedule. Control groups were housed 6 to the same cage without partitions. M1 receptors were evaluated by Pirenzepine binding while choline uptake by HC-3, choline acetyltransferase according to (Lau et al, 1987) acetyl cholinesterase by (Ellman et al, 1961 and Fatranska et al, 1987), M1 mRNA and protein by Northern and Western blotting respectively. **Results:** DR to 40% significantly decreased choline uptake ($p < 0.05$) and M1 receptor number (B_{max}) ($p < 0.05$), without changes in affinity (K_d), choline acetyltransferase (ChAT) or acetyl cholinesterase (AChE) activity. Tyrosine administration significantly increased choline uptake ($p < 0.05$) and M1 density in the 40% DR ($p < 0.01$) without changes in affinity. ChAT activity was decreased after tyrosine-significantly after 40% DR ($p < 0.05$) while AChE was not affected. M1 mRNA and protein were not influenced by DR or tyrosine. Separation stress increased M1 receptor density ($p < 0.05$) and its mRNA signal ($p < 0.001$), without changes in choline uptake, ChAT and AChE. Tyrosine further increased M1 receptor density of stressed mice ($p < 0.05$). **Conclusion:** Tyrosine might be a potential therapy for the management of problems associated with stress induced weight loss.

THE EFFECT OF TYROSINE ON COGNITIVE FUNCTION IN ANIMAL MODELS FOR ANOREXIA NERVOSA

D. Ben Shushan, Y. Avraham, S. Hao, S. Mendelson, E.M. Berry

Department of Human Nutrition and Metabolism, Hebrew University of Jerusalem, Hadassah Medical School, Jerusalem, Israel

Background: We have investigated the effect of tyrosine administration (100mg/kg) on cognitive function in models of anorexia in mice, associated with either Diet Restriction (DR), Separation Stress (S) or Activity (A), which mimic some of the behavioral symptoms of patients with Anorexia Nervosa (AN). **Methods:** In the DR model, young female Sabra mice were fed 100, 60 and 40% of the calculated daily nutritional requirements for a period of 18 days. In the Separation model, the mice were housed in cages fitted with six individual plexiglas partitions; they could see each other without any physical contact except when transferred to regular cages for two hours feeding. In the Activity model, mice were placed 2 in a cage with a fixed wheel attached to an electronic revolution counter. Feeding schedule was the same as in the Separation model. In all models, mice were divided into 4 groups of 10 mice: control and treatment groups with and without tyrosine. Cognitive function was evaluated using the eight-arm maze or the T-maze. **Results:** DR: animals fed to 60% showed significantly improved maze performance while it was impaired in animals on 40% DR. Administration of tyrosine restored the maze performance. **Separation:** There was a decrease in spontaneous alternations in the separated animals, which was restored by tyrosine to control levels. **Activity:** the activity group performed better than controls in the 8-arm maze, and this increased further after tyrosine. **Conclusions:** Tyrosine treatment improves some of the neurobiological disturbances caused by DR, Separation and Activity. Therefore, tyrosine might be a potential therapy for cognitive problems associated with the maintenance of reduced body weight, after prolonged dieting and also in the treatment of AN patients.

EFFECTS OF LITHIUM CHLORIDE ON MEMORY PERFORMANCES OF MICE IN ELEVATED PLUS-MAZE TEST

P. Yamanturk, L. Eroglu

Istanbul University, Faculty of Medicine, Department of Pharmacology and Clinical Pharmacology, Istanbul, Turkey

It has been frequently reported that cognitive functions of patients use lithium are impaired although there is an inconsistency in this situation. Therefore the effects of lithium chloride (LiCl) on memory performances of mice were investigated in elevated plus-maze test. In this test transfer latency (TL) the time it took for each mouse to move from the open arm to either of the enclosed arms where feels itself safe is recorded twice in 24 hours. On second trial day, learning leads a shortening in TL values and its prolongation indicates memory impairment. Neither acute nor chronic LiCl administrations have altered memory performances of mice. The results suggest that lithium may not always affect memory as negative. Besides scopolamine, an anticholinergic agent induced prolongation of TL was reversed by LiCl. So it can be considered that lithium may ameliorate some kinds of memory impairments and the cholinergic system may be involved in its actions on memory.

EFFECTS OF 7-NITROINDAZOLE ON MEMORY PERFORMANCES OF RATS TRAINED FOR THREE-PANEL RUNWAY TASK: HIPPOCAMPAL CHOLINERGIC ENZYME ACTIVITIES

P. Yamanturk¹, Y. Unlucerci², S. Bekpinar², H. Koyuncuoglu³

¹Istanbul University, Istanbul Faculty of Medicine, Department of Pharmacology and Clinical Pharmacology, Capa, Istanbul, Turkey,

²Istanbul University, Istanbul Faculty of Medicine, Department of Biochemistry, Capa, Istanbul, Turkey, ³Istanbul University, Institute of Experimental Medicine, Department of Neuroscience, Sehremeni, Istanbul, Turkey

Nitric oxide synthase (NOS) inhibition leads memory deficits in some animal experiments and does not impair memory in some others. Roles of cholinergic and nitrergic systems in working and reference memory performances of rats were assessed regarding the relation between them. Animals trained for working and reference memory performances in three-panel runway were injected intraperitoneally with selective neuronal NOS inhibitor, 7-nitroindazole (7-NI) and observed 45 minutes later from this administration in the runway recording error (wrong door choosing) and latency (time to reach the food pellet in goal box). The activities of choline acetyltransferase (CAT) and acetylcholinesterase (AChE) were measured in hippocampi of rats taken immediately after the test. The results have showed that systemic administration of 7-NI did not affect the both kinds of memory performances and there was not any alteration in hippocampal CAT and AChE activities in rats administered NOS inhibitor comparing control group. In conclusion, nitric oxide does not seem to contribute to mechanisms underlying memory performances of rats under these experimental conditions and the results suggest that the cholinergic system is not involved in the absence of the possible impairing effects of NOS inhibition on memory.

This work was supported by the Research Fund of the University of Istanbul (Project Number: 1014/250897)

HYDROCORTISONE AFFECTS THE DENSITIES OF CARDIAC MUSCARINIC AND ADRENERGIC RECEPTORS

Jaromir Myslivecek^{1,2}, Jan Rícný¹, Stanislav Tucek¹

¹Institute of Physiology, Academy of Sciences of the Czech Republic,

²Institute of Physiology, Charles University, First Medical Faculty, Prague, Czech Republic

Glucocorticoid hormones affect the expression of a number of proteins but information on their effects on neurotransmitter receptors in the heart is incomplete and controversial. We investigated the effects of repeated administrations (1-12 days) of high doses of hydrocortisone (50 mg/kg) to adult rats on the densities of cardiac muscarinic and alpha-1, beta-1, beta-2 and putative beta-4 adrenergic receptors, on the coupling of muscarinic receptors with G proteins and on the control of adenylyl cyclase.

Hydrocortisone enhanced the densities of muscarinic receptors in the atria and both ventricles. The density of beta-1 adrenoceptors became enhanced in the atria, and that of beta-2 adrenoceptors was raised in the atria and ventricles. The density of putative beta-4 adrenoceptors first increased and then diminished in the atria and did not change in the ventricles. It varied independently of the density of beta-1 adrenoceptors, although the putative beta-4 adrenoceptors are now proposed to represent an atypical state of beta-1 adrenoceptors. The alpha-1 adrenoceptors underwent a decrease followed by a transient increase in the atria and no change in the ventricles. According to the effects of GTP on the binding of carbachol, the coupling of muscarinic receptors with G proteins was not affected. The activity of adenylyl cyclase (determined by HPLC) and its stimulation by isoprenaline and inhibition by carbachol were not significantly altered.

The findings raise the question of possible roles of glucocorticoids in the control of the expression of neurotransmitter receptors in healthy and diseased hearts.

ACETYLCHOLINE AND NO-MEDIATED CGMP SYNTHESIS IN THE RAT BRAIN

W.C.G. van Staveren, M. Markerink-van Ittersum, H.W.M. Steinbusch, J. de Vente

Maastricht University, Department of Psychiatry and Neuropsychology, Maastricht, The Netherlands

About 30 years ago acetylcholine was linked to cGMP in the brain for the first time. Only recently it was shown that NO-mediated cGMP synthesis takes place in cholinergic fibers in the forebrain of the rats (De Vente et al. Exp. Brain Res. 136, 480-491 2001). NO - cGMP signal transduction is found throughout the central nervous system. The NO stimulated intracellular increase in cGMP will be short lived due to the presence of phosphodiesterases (PDE). PDE activity in the the brain is generally very high. PDE's present a very complex group of enzymes, containing a large number of isoforms classified into 11 subfamilies. Each family has its own structural characteristics, substrate preference and inhibitor profile. It is not known which PDE isoforms are present and in control of cGMP levels in cholinergic neurons. We incubated brains slices in vitro in the presence of different PDE inhibitors and different NO donor compounds. cGMP synthesizing structures were visualized and characterized using immunocytochemistry. cGMP synthesis was found to be present virtually all of cholinergic fibers in the cerebral cortex and in almost all cholinergic fibers of the basal ganglia. Further details will be presented on the presence of PDE isoforms in these cholinergic fibers.

EEG EVALUATION OF HUPERZINE A, A REVERSIBLE CHOLINESTERASE INHIBITOR

S.L. Hale¹, H. Ved², A. Williams³, B. Doctor², F. Tortella³

¹Division of Pathology¹, Division of Biochemistry², Division of Neurosciences³, Walter Reed Army Institute of Research, Silver Spring, MD, USA

Huperzine A (HupA), an alkaloid isolated from the Chinese club moss, *Huperzia serrata*, is characterized as a potent, reversible cholinesterase inhibitor that crosses the blood brain barrier. This compound has previously been shown to provide protection against seizures, neuropathology and mortality induced in laboratory animals by subsequent challenge with organophosphate nerve agents (i.e. soman). Because of HupA's potential as a neuroprotective drug, identification of potentially serious CNS side effects is a necessary part of its evaluation. In this study the EEG and behavioral effects of a single i.v. injection of HupA (0.5-2.0 mg/kg, n=5/dose) were evaluated over a 24 hour period. Male Sprague-Dawley rats were implanted with cortical EEG electrodes and jugular vein catheters several days prior to administration of HupA. Immediately following treatment, dose dependent symptoms of muscle fasciculation, intense sedation and a significant delay in the onset to normal EEG slow-wave sleep (maximum = 167 ± 10 min/n=10) were apparent. There was no evidence of seizure activity or marked cortical slowing. However, at the three highest doses of HupA evaluated (1.5, 1.75, 2.0 mg/kg), computer-assisted spectral analysis revealed significant shifts in the EEG frequency pattern relative to baseline controls. EEG power increased in the 4-8 Hz frequency band with dose-dependent consolidation of the EEG waveform centered at a peak frequency of 5.5 Hz. Light microscopic evaluation of HupA treated brains removed 24 h postinjection failed to reveal histological evidence of lesions. In rats, even near-lethal doses of the cholinesterase inhibitor HupA were apparently without significant functional or pathological central nervous system intoxication.

HUPERZINE A AND CHOLINESTERASE INHIBITORS: GLUTAMATE AND BENZODIAZEPINE RECEPTOR INTERACTIONS

S.V. Nigam, B.P. Doctor, H.S. Ved, R.K. Gordon

*Division of Biochemistry, Walter Reed Army Institute of Research,
Silver Spring, MD, USA*

Huperzine A (HupA) is a natural alkaloid that exhibits a unique dual pharmacology: it is a potent and specific acetylcholinesterase inhibitor over butyrylcholinesterase, but also a neuroprotective agent. We demonstrated that HupA protected primary neuronal cells against excitatory amino acid induced neurotoxicity. Part of its pharmacological action resides in its ability to non-competitively inhibit the passage of calcium ions through NMDA ion channels. Thus, we established that HupA non-competitively inhibited 3H-MK801 and 3H-TCP binding to synaptosomal plasma membranes, (pseudo K_i of 5-10 μ M). MK801 and TCP are specific probes for the NMDA ion-channel PCP site. HupA did not interact at the NMDA glycine, polyamine, or agonist sites as determined by ligand binding studies. In addition, similar results were observed for the (+)stereoisomer of HupA, which exhibits markedly reduced anticholinesterase activity. Additional evidence that the neuroprotective effects of HupA were separate from cholinesterase activity is that cholinesterase inhibitors such as tacrine, physostigmine, or E2020 showed markedly less effect in displacing 3H-MK801. Unlike MK801, HupA, in the presence of an irreversible cholinesterase inhibitor, dissociated from rat brain NMDA receptors in synaptosomal plasma membranes with both a fast and slow component. This may be another advantage of HupA over high affinity ion-channel blockers that exhibit side effects such as PCP. HupA also exhibited antagonism of central benzodiazepine receptors measured with 3H-RY-80, although it was less strong than observed for 3H-MK801. These data suggest that neuroprotection by HupA against excitatory-induced cell death is at least partially based on the blockage of calcium ions through NMDA ion channels, and that the effect is independent of its efficacy as a cholinesterase inhibitor.

CHOLINERGIC MODULATION OF CHEMOTAXIS IN HUMAN MELANOMA CELLS

A. Boss, S. Noda, M. Sailer, M. Oppitz, U. Drews

Institute of Anatomy, University of Tübingen, Germany

Neural crest cells transiently express muscarinic acetylcholine receptors during migration from the neural tube to the definitive location in the epidermis. Differentiated melanocytes of normal human skin do not express muscarinic receptors. After malignant transformation receptors re-appear in primary and metastatic human melanomas. We assume that an embryonic trait which is involved in morphogenesis, is reactivated in a pre-malignant or malignant state and mediates cellular movements during invasive growth. For further characterization, we used the human melanoma cell line SK-Mel 28. Pharmacological characterization via dose-response curves of calcium mobilisation after stimulation by acetylcholine indicated expression of the M3 subtype with a K_D of approximately 2×10^{-6} M. Molecular weight of muscarinic receptors of SK-Mel 28 cells determined by western blot with M3 subtype specific antibodies was 66kDa which is in accordance to published data for the M3 subtype. In time lapse video-microscopy, stimulation of muscarinic receptors with acetylcholine and carbachol induced contractions and a change in cell shape. In the present study, an effect on chemotactic activity by cholinergic treatment is demonstrated. By chemotaxis assays with modified Boyden chambers, an increase of movement towards the chemotactic factor fibronectin was found after addition of carbachol in the upper compartment. This indicates a modulatory effect on cell movement of the muscarinic cholinergic system in non neural cells.

CALCIUM MOBILISATION AND CELLULAR CONTRACTION OF EMBRYONIC LENS VESICLE AND NEURAL TUBE ON MUSCARINIC CHOLINERGIC STIMULATION

U. Drews, M. Oppitz, G. Schrick

Institute of Anatomy, University of Tübingen, Germany

Embryonic epithelial structures like the neural tube and the lens vesicle exhibit cholinesterase activity during phases of morphogenetic movements. Cholinesterase activity coincides with the expression of muscarinic receptors. Here we show that the isolated lens vesicle and the intact neural tube in the chick embryo react on muscarinic cholinergic stimulation by calcium mobilisation and concomitant cellular contraction. The lens vesicle was stained with Fura2-AM and studied by digital time lapse epifluorescence video. On stimulation with carbachol we observed a peak of intracellular calcium release followed by a plateau phase of extracellular influx, which was reversed by addition of atropine. The peak reaction was accompanied by contraction of the lens. Addition of atropine led to relaxation to the initial diameter. Experiments with the neural tube were performed by perfusion of the neural canal in the intact embryo. After perfusion with atropine a characteristic, but reversible deformation of the neural tube occurred. The experiments demonstrate, that the embryonic muscarinic system is involved in cellular movements of non-neural cells during morphogenesis.

COMBINED ANDROGEN-DONEPEZIL TREATMENT IN POST-STROKE REHABILITATION

John W. Crayton, Lukasz M. Konopka, Alexander G. Karczmar

*Biological Psychiatry Section, Hines VA Hospital and Loyola Stritch School
of Medicine, Maywood, Illinois, USA*

There is a theoretical basis for thinking that a combination of androgenic steroid DHEA and the anti-cholinesterase, donepezil may have an enhanced (greater than additive) effect in the post-stroke syndrome. Steroids increase potassium conductance in central neurons and in particular, hippocampal neurons (eg. Colino and Halliwell, *Nature* 328:73, 1987). Under certain circumstances, this effect leads to hyperpolarization and a modulatory effect on neurotransmitter function (Beck et al., *Neuropsychopharmacol.* 14:27, 1996). More specifically, androgens have been shown to facilitate the responses of hippocampal neurons to cholinomimetics (including ACh's). In fact, Karczmar and Dun showed that androgens enabled non-cholinergic neurons to respond to the direct actions of cholinergic agents. (Karczmar and Dun, unpublished observations) Androgenic steroids produce trophic in several systems, including neuronal systems (Jones, *NY Acad Sci* 743:141, 1994) They improve muscle strength, mood and memory, particularly in elderly, hypo-gonadal subjects. (Tenover, *J Clin Endocrinol Met* 75:1092, 1992) Taken together, these considerations suggest that DHEA will enhance the beneficial effects of donepezil in this population and hence speed recovery from stroke. We will describe a study which aims to determine if the hypothesized synergy between androgens and donepezil promotes enhanced recovery from stroke. Patients with recent strokes receive donepezil alone, DHEA alone, or a combination of the two agents. They are followed over a six-month period with monitoring of their neurological, neuropsychological, and psychological functioning. In addition, patients will be followed with repeated MRI and EEG studies. Preliminary data from this ongoing study will be presented.

We acknowledge the support of the Illinois AMVETS and the Chicago Association for Research and Education in this work.

LOCALISATION OF THE HIGH-AFFINITY CHOLINE TRANSPORTER-1 IN RAT SKELETAL MUSCLE AND SPINAL CORD

K.S. Lips, U. Pfeil, R.V. Haberberger, W. Kummer

Institute for Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany

Acetylcholine (ACh) is synthesised by choline acetyltransferase in the cytoplasm of cholinergic neurons. The vesicular acetylcholine transporter (VACHT) imports ACh into synaptic vesicles. After exocytotic release it is split into acetate and choline. Choline is taken up into the synaptic terminal via a high-affinity choline transporter (CHT) for resynthesis of ACh. The first high-affinity CHT (CHT1) was recently cloned, and in-situ hybridization showed its expression in spinal motoneurons. We generated polyclonal antisera against a synthetic peptide corresponding to aa residues 29-40 of the rat CHT1 sequence. These antisera were used in immunofluorescence to analyse the distribution of the CHT1 protein either singly or in combination with antisera against VACHT to label cholinergic terminals, and Alexa-488 conjugated α -bungarotoxin to label motor end plates. Perikarya of spinal motoneurons were moderately CHT1- and VACHT-immunoreactive, while the recurrent cholinergic synapses were intensely CHT1- and VACHT-immunolabelled. In skeletal muscles, motor end plates showed an intense CHT1- and VACHT-immunoreactivity, while preterminal axons showed a distinct CHT1- but only weak VACHT-immunolabelling. The results show a preferential localisation of the CHT1 protein at the neuro-neuronal and neuro-muscular synapse, well in line with its anticipated function in the synaptic transmitter recycling. (supported by the DFG, SFB 547).

HUPERZINE A, A PROMISING ANTI-ALZHEIMER'S AGENT, REDUCES STAUROSPORINE-INDUCED APOPTOSIS IN NG108-15 CELLS

Yi-Fan Han¹, Xiao-Qiu Xiao², Dong-Cheng Wu¹, Yang Gao¹, Wing-Lok Ho¹, Nelson Tze-Kin Lee¹, Yan Fu², Karl Wah-Keung Tsim¹

¹Hong Kong University of Science and Technology, Hong Kong, China.

²State Key Laboratory of Drug Research, Shanghai Institute of Material Medica, Shanghai Institute of Life Science, Chinese Academy of Sciences, Shanghai, China

The present study investigates the effects of huperzine A (HupA) and tacrine (Tac), two anti-Alzheimer's agents, on staurosporine-induced apoptosis and potential mechanisms in neuroblastoma hybride NG108-15 cell lines. Preincubation with HupA and Tac significantly attenuated 0.1 μ M staurosporine-induced chromatin condensation, nuclei fragmentation and DNA laddering, and inhibited or delayed expression of the pro-apoptotic gene product Bax, and increased Bcl-2 levels. Acetylcholinesterase (AChE) enzymatic and Western blot assay demonstrated that staurosporine treatment induced an abnormal AChE activity that was resistant to HupA and Tac. These results suggest that HupA and Tac might exert significant protection against staurosporine-induced apoptosis via suppressing the abnormal AChE and modulating expression of apoptosis-related proteins in apoptotic cells, which might be beneficial for its therapeutic usage.

Acknowledgements: Sincere thanks are extended to Profs. Xi-Can Tang, Israel Silman and Stephen Brimijoin for their advice on this project.

CHOLINERGIC-GLUTAMATERGIC INTERACTIONS IN HIPPOCAMPAL NEURONS: POSSIBLE ROLE IN THE NORMAL AND DISEASED HIPPOCAMPUS

Lev Pavlovsky, Alon Friedman

Departments of Physiology and Neurosurgery, Ben Gurion University and Soroka Medical Center, Zlotowski Center of Neuroscience, Beersheva, Israel

In the cortex, released Acetylcholine (ACh) binds to both pre- and post-synaptic receptors and assumed to serve as a modulatory neurotransmitter and thus set the response of the nerve cell to an incoming stimulus. The electrophysiological details of such modulation are only partly understood. We have tested the role of ACh on excitatory synaptic transmission in the CA1 area of the hippocampus, using extracellular and intracellular recordings. In whole cell voltage clamp experiments, the frequency, but not amplitude, of spontaneously occurring excitatory synaptic currents (EPSCs) was increased following either local application of ACh or the addition of cholinesterase inhibitors (ChEIs) to the bathing solution. In contrast, addition of the muscarinic antagonist, atropine, reduced EPSCs frequency, suggesting a role for basal ACh release in modulating glutamate transmission. The effect of ChEIs on the local neuronal network was tested using extracellular recordings of evoked responses. In control animals following ChEIs exposure a mild (<2 fold) increase in the amplitude of population spikes was observed. In contrast, weeks following stress, or injections of low concentrations of the AChEI, di-isopropyl-fluorophosphonate (DFP), a marked sensitivity of the evoked synaptic response to either AChEIs or Atropine was noted. These results suggest that ACh-regulated synaptic transmission might alter in response to environmental conditions, and be associated with marked alterations in cortical function.

DOPMINE RELEASE FROM RAT STRIATAL SLICES IN VITRO AND FUNCTIONAL EFFECTS IN 6-OHDA TREATED RATS IN VIVO ARE MEDIATED BY BETA2 CONTAINING NICOTINIC ACETYLCHOLINE RECEPTORS

T.K. Murray, F.A. Jones, D. Steggles, D.R. Dobson, C.P. Dell, I.A. Pullar, M.J. O'Neill

Eli Lilly and Co. Ltd., Windlesham, Surrey, UK

Several studies have reported that nicotinic acetylcholine receptors (nAChRs) are located on dopaminergic cell bodies and terminals and that activation of neuronal nAChRs plays a role in the reinforcing properties of nicotine and cigarette smoking. It is also clear that there is a negative correlation between smoking and the incidence of Parkinson's Disease and that nicotine can increase dopamine release in vitro and in vivo. We have utilised several new ligands for nicotinic receptor sub-types to investigate the nAChRs involved in dopamine release in vitro and the functional effects of dopamine in vivo. We have evaluated the effects of SIB-1508Y and TC-2559 (beta2 preferring), nicotine (a broad spectrum agonist), SIB-1553A (beta4 preferring) and Astra II (alpha7 selective) on dopamine release from rat striatal slices and on rotational behaviour in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Nicotine, TC-2559 and SIB-1508Y produced a dose-dependent, mecamylamine sensitive increase in dopamine release in vitro. In contrast, SIB-1553A and Astra II did not stimulate dopamine release. In vivo, SIB-1508Y (10 mg/kg s.c), nicotine (0.5 mg/kg s.c) and TC-2559 (5 mg/kg) produced a significant increase in ipsiversive rotations ($p < 0.05$), while SIB-1553A (20 mg/kg s.c) and Astra II (10 mg/kg s.c) had no effect. The SIB-1508Y-induced increases in rotational behaviour were blocked by mecamylamine. These results indicate that dopamine release in vitro and in vivo is mediated by beta2 containing nicotinic receptor sub-types and suggest that beta2 nAChR agonists may provide a useful symptomatic treatment for Parkinson's Disease.

THE EFFECTS OF GALANTAMINE IN PATIENTS WITH REFRACTORY SCHIZOPHRENIA RECEIVING RISPERIDONE

J.P. McEvoy, T.B. Allen

Duke University Medical Center, Durham, NC, USA

Objective: The prevalence of smoking in patients with schizophrenia is higher than in the general population. Some schizophrenia patients may have abnormal nicotinic neurophysiology, since sensory gating deficiencies can be corrected by nicotine. Galantamine (Reminyl(R); GAL) is a reversible acetylcholinesterase inhibitor (AChEI) and an allosteric modulator of nicotinic acetylcholine (ACh) receptors (nAChR), for treating mild-to-moderate Alzheimer's disease. This ongoing 4-week, dose-finding study aims to investigate the effect of GAL on behaviour in risperidone (RIS)-treated patients with schizophrenia. **Methods:** All patients are being treated with fixed-dose RIS (1-6 mg daily). Patients (n = 24) are divided into 4 groups. Groups 1-3 have 2 patients on placebo (PLA) and 6 patients on GAL (Group 1: 8 mg BID, Group 2: 12 mg BID, Group 3: 16 mg BID); standard dose-escalation is undertaken for GAL. All patients in Group 4 receive PLA. Psychopathology is assessed using the Brief Psychiatric Rating Scale and Clinical Global Impression at baseline and at days 7, 14, 21 and 28. Cognitive psychomotor performance, neuropsychological functioning and smoking behaviour are also assessed at varied time-points. **Results:** In two patients with refractory schizophrenia, preliminary findings with GAL have shown improvement in episodes of agitation in one patient (8 mg BID) and improved social and hygiene manners in another patient (12 mg BID). **Conclusion:** The results from this ongoing study and subsequent studies may aid in establishing a therapeutic use for GAL in patients with refractory schizophrenia.

THE ROLE OF LIPID PEROXIDATION IN THE MECHANISM OF NEUROTOXICITY OF ORGANOPHOSPHATES

V.D. Tonkopii

Institute of Limnology, Russian Academy of Sciences, St. Petersburg, Russia

The present study was undertaken to elucidate the relations between lipid peroxidation, organophosphates (OPs) toxicity and delayed, long lasting, non-cholinergic changes. In the experiments on the rats we studied the influence of OPs intoxication by paraoxon, sarin, malathion, soman on lipid peroxidation in rat cerebral hemispheres. The level of lipid peroxidation was measured as the amount of common phospholipids, peroxidate lipids and malondialdehyde (MDA) in reaction with thiobarbituric acid. Results were compared to those with pre-treatment with atropine and reversible cholinesterase inhibitor - galanthamine alone or together with different antioxidants (tocopherol, oxymetacyl, ionole). The rate of reaction of conditioned reflex of active avoidance was measured. OPs caused a rapid, dose-dependent increase of peroxidate lipids and MDA 15-30 days after intoxication. With paraoxon and sarin pre-treatment with atropine and galanthamine totally prevents the all symptoms of intoxication and changes in lipid peroxidation. Comparatively such type of prophylaxis in malathion and soman poisoned rats didn't normalize the biochemical and physiological parameters. The protective effect of antioxidants against soman and malathion - induced lipid peroxidation was shown. Therefore, malathion and soman - associated lipid peroxidation is likely to arise mainly as a primary change which may, however, play a significant role in delayed neurotoxicity and conditioned reflex activity.

CEREBRAL METABOLIC ACTIVATION WITH CHOLINESTERASE INHIBITOR THERAPY IN ALZHEIMER'S DISEASE

M. Mega¹, I.D. Dinov¹, M. Manese¹, J. Felix¹, S.M. O'Connor¹, J.L. Cummings², A.W. Toga¹

¹Laboratory of Neuroimaging, ²Department of Neurology, Reed Neurological Research Center, Los Angeles, CA, USA

Objective: The clinical response of patients with Alzheimer's disease (AD) to cholinergic treatment is similar with different cholinesterase inhibitors, suggesting a common neuronal system affected by general cholinergic augmentation. We performed a study to compare functional patterns of metabolic response and identify brain regions affected by increased cholinergic tone due to treatment with various cholinergic AD treatments. **Methods:** Demographically similar mild AD patients (n = 12) treated either with metrifonate, donepezil or galantamine (Reminyl(R)) were studied from a pool of 30 patients. Pre- and post-treatment [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) was performed, and patients were followed using the Mini-Mental State Examination and the Neuropsychiatric Inventory. Patients' normalised PET studies, registered to the AD probabilistic anatomic atlas, were subjected to a voxel-by-voxel subtraction of the post-treatment minus pre-treatment studies. Sub-volume thresholding was used to correct random lobar noise, allowing the production of 3-D functional significance maps for total brain voxel comparison. **Results:** Significant post-treatment anterior cingulate, dorsolateral frontal, and supramarginal activation was observed with cholinergic treatment in pooled groups. Different mechanisms of action of the AD treatments studied could underlie differential activation patterns among the patient groups. More results will be presented that may establish this further. **Conclusion:** FDG-PET has potential as a useful technique to enhance our understanding of possible differences in the effects a number of cholinergic treatments in the brains of patients with AD. For instance, attentional networks, with greatest activation centred on the anterior cingulate, appear to be a key neurophysiological target activated by cholinergic AD treatments.

RESCUE OF THE NEURODEGENERATIVE PHENOTYPE IN AD11 ANTI-NGF MICE

S. Capsoni¹, S. Giannotta¹, A. Cattaneo²

¹International School for Advanced Studies (SISSA), Italy,

²Institute of Neurophysiology, CNR, Pisa, Italy

We have obtained transgenic mice expressing a neutralizing anti-NGF recombinant antibody, in which the levels of antibodies are three order of magnitude higher in adults than in newborns (Ruberti et al., J. Neurosci. 20, 2000). The analysis of brains of these mice revealed that they display a progressive neurodegeneration characterized by neuronal loss, cholinergic deficit, tau hyperphosphorylation, extracellular deposits of amyloid precursor protein (APP) and behavioral deficits (Capsoni et al., Proc. Natl. Acad. Sci. USA 97, 2000). Beta-amyloid cerebrovascular deposition and beta-amyloid plaques are observed as well (Capsoni et al., submitted). In this study, we performed pharmacological treatments of AD11 anti-NGF mice with acetylcholinesterase inhibitors and agents that increase NGF levels and/or activity. Different time windows were examined. Amelioration of the neurodegenerative phenotype was achieved both with acetylcholinesterase inhibitors and NGF, thus showing the dependency of the phenotype from NGF deprivation. This study was partially supported by Telethon (grant D.122) and SIRS s.r.l.(now Lay Line Genomics S.p.A.)

ACUTE CHOLINERGIC RESCUE OF SYNAPTIC PLASTICITY IN THE NEURODEGENERATING CORTEX OF ANTI-NERVE GROWTH FACTOR MICE

E. Pesavento¹, S. Capsoni¹, L. Domenici^{1,2}, A. Cattaneo¹

¹International School for Advanced Studies (SISSA), Italy.

²Institute of Neurophysiology, CNR, Pisa, Italy

Deficits in cholinergic systems innervating cerebral cortex are associated to cognitive impairment during senescence and in age-related neurodegenerative pathologies. However, little is known about the role of cholinergic pathways in modulating cortical plasticity. Basal forebrain cholinergic neurones are a major target for Nerve-Growth Factor (NGF). In order to investigate the relationship between cholinergic innervation and cortical synaptic plasticity, we exploited a transgenic mouse model in which the activity of NGF in the adult nervous system is neutralized by the expression of blocking antibodies to NGF itself (anti-NGF mice) [Ruberti, F., et al. (2000). *J. Neuroscience*, 20, 2589-2601]. In six months old anti-NGF mice, we show that the reduction in cholinergic innervation of the cortex is associated to different forms of synaptic plasticity impairment. A local, acute increase in the availability of acetylcholine rescues these synaptic plasticity deficits, thus indicating that cholinergic system mediates the impairment of cortical plasticity at this early stage of the neurodegenerative process triggered by NGF neutralization. Our results represent an important step to unveil the pivotal role of cholinergic transmission in modulating adult cortical plasticity. This study was partially supported by Telethon (grant D.122) and SIRS s.r.l. (now Lay Line Genomics S.p.A.).

A PEPTIDE FROM THE C-TERMINAL OLIGOMERISATION DOMAIN OF HUMAN SYNAPTIC (T-FORM) ACETYLCHOLINESTERASE FORMS CLASSICAL AMYLOID FIBRILS

M.G. Cottingham¹, M.S. Hollinshead¹, D.J.T. Vaux¹

¹Sir William Dunn School of Pathology, Oxford University, UK

Acetylcholinesterase (AChE) is abnormally localised in amyloid plaques, which are a key neuropathological feature of Alzheimer's disease. It has recently been shown to interact in vitro with the beta-amyloid peptide, which is the principal constituent of the Alzheimer plaques, and to increase the rate of its assembly into amyloid fibrils (Alvarez, 1997 & 1998; Inestrosa, 1998). We report that a synthetic peptide corresponding to residues 586 to 599 of the synaptic or tailed (T) splice variant of human AChE can itself form typical amyloid fibrils similar to those formed by beta-amyloid. Under physiological buffering conditions, the peptide rapidly aggregates into fibrillar structures that have all the classical features of amyloid. Electron microscopical analysis of negatively stained aggregates reveals that they are composed of long fibrils which are 6-7nm in diameter. The fibrils bind both Congo Red and thioflavin-T and cause the expected metachromatic shift in the absorbance spectrum of Congo red and in the fluorescence excitation spectrum of thioflavin-T. Far-UV circular dichroism spectroscopy reveals that fibril formation by the peptide is accompanied by a change in its secondary structure from random coil to beta-sheet. Amyloid fibrils have a generic 'cross-beta' structure consisting of repeating beta-sheets running perpendicular to the fibril axis. Furthermore, the peptide is cytotoxic in vitro, as determined by its effect upon the reduction of MTT by PC-12 pheochromocytoma tissue culture cells. This fibrillogenic region of the C-terminal oligomerisation domain of T-form AChE might be responsible for an interaction of the enzyme with beta-amyloid.

THE EFFECT OF NICOTINE ON EXPRESSION OF NICOTINIC RECEPTORS IN THE BRAIN OF PATIENTS WITH ALZHEIMER'S DISEASE

M. Mousavi¹, E.L. Hellstrom-Lindahl¹, Z.-Z. Guan¹, K.-R. Shan¹, R. Ravid², A. Nordberg¹

¹Ki, NEUROTEC, Karolinska Institute, Department of NEUROTEC, Division of Molecular Neuropharmacology, Huddinge University Hospital, Stockholm, Sweden, ²Netherlands Brain Bank, Amsterdam, The Netherlands

Epidemiological studies have suggested some association between smoking and Alzheimer's disease (AD). Nicotine treatment may compensate for some of the cholinergic deficits e.g. decreased number of nicotinic acetylcholine receptors (nAChRs) seen in AD. In the present study the effect of smoking on nAChRs in AD was investigated in autopsy brains using receptor binding and Western blotting techniques. The nAChR subtypes were measured using radioactive ligands 3H-cytisine (alpha 4), 3H-epibatidine (alpha 3, 4) and 125I- aBTX (alpha 7). The binding of 3H-cytisine (2.0 nM) and 3H-epibatidine (0.1 nM) was significantly increased in the hippocampus, cerebellum, frontal and temporal cortices and 125I- aBTX (3.5 nM) in the temporal cortex of smoking controls (SC) (69.5±3.2 years; n=4-11) compared to non-smoking controls (NSC) (78.9±3.5 years, n=9-10). A significantly increased binding of 3H-epibatidine and 3H-cytisine in the temporal cortex and 3H-cytisine in the cerebellum was observed in smoking AD (SAD) (73.3±3.5 years, n=8-13) compared to non-smoking AD (NSAD) (80.2±3.2 years, n=8-13). Binding levels for both ligands in SAD was similar to those in NSC. Up-regulation of 3H-cytisine, 3H-epibatidine and 125I- aBTX binding in the temporal cortex of SC compared to NSC was positively correlated with an increased level of alpha 3, 4 and 7 protein expression. This study showed an increased number of nAChRs, especially alpha 4 in SAD compared to NSAD, which may be relevant to a neuroprotective effect of nicotine.

NICOTINE INDUCES GLUTAMATE RELEASE FROM HIPPOCAMPAL MOSSY FIBRES SYNAPTOSOMES

V. Bancila, A. Bloc, Y. Dunant

Departement de Pharmacologie, CMU, Geneva, Switzerland

Nicotine is known to modulate the release of several neurotransmitters from mammalian CNS synaptosomes: Dopamine, ACh, Noradrenaline and GABA. In addition, electrophysiological evidence suggests that nicotine also promotes the release of glutamate. In the present work glutamate release was elicited from rat hippocampal mossy fibre synaptosomes by using nicotinic agonists, and measured by a luminescence assay. Glutamate release was elicited by micromolar concentrations of nicotine. The maximal effect was obtained with 25 mM nicotine, and blocked at 90% of control by curare 100 mM. The effects of other antagonists is presently under investigation. In parallel experiments, carbocyanide fluorescence was used to measure the membrane potential. When promoting glutamate release, nicotine did not induce significant depolarisation. Nonetheless, the amount of transmitter released by maximal doses of nicotine corresponded to that induced by approximately 25 mM KCl. We are currently investigating the pharmacological profile of mossy fibres presynaptic receptors.

Supported by the FNRS Grant # 31 57135 99

NICOTINIC CHOLINERGIC ACTIVATION OF MAGNOCELLULAR ENDOCRINE NEURONS OF THE HYPOTHALAMUS

M. Zaninetti¹, E. Tribollet², D. Bertrand², R. Ogier², M. Raggenbass²

¹Ophthalmology Clinic, Geneva University Hospitals, ²Department of Physiology, University Medical Center, Geneva, Switzerland

Using IR DIC videomicroscopy, we performed whole-cell recordings in hypothalamic slices containing the supraoptic (SO) and paraventricular (Pa) nuclei. Acetylcholine (ACh), locally applied in the presence of atropine, evoked a rapidly rising inward current in SO and Pa magnocellular neurons, which persisted in the presence of blockers of synaptic transmission. It could be reversibly suppressed by methyllycaconitine, a selective antagonist of $\alpha 7$ -containing nicotinic receptors, but was insensitive to dihydro-beta-erithroidine, an antagonist acting preferentially on non- $\alpha 7$ nAChRs. The effect of ACh could be mimicked by (-)-2PABH, a recently synthesized nicotinic agonist specific for $\alpha 7$ nAChRs. ACh also desensitized nicotinic receptors. Desensitization was pronounced and recovery from desensitization was rapid, consistent with the properties of $\alpha 7$ -containing nAChRs. Nicotinic currents could not be evoked in Pa parvocellular neurons, suggesting that these neurons are devoid of functional nicotinic receptors. Light microscopic autoradiography showed that [125I]alpha-bungarotoxin binding sites are present in the SO and in all magnocellular divisions of the Pa, but are undetectable in other areas of the Pa. Immunohistochemistry, performed using antibodies directed against vasopressin and oxytocin, indicated that responsiveness to nicotinic agonists was a property of vasopressin as well as of oxytocin magnocellular neurons, in both the SO and Pa nuclei. In conclusion, by directly increasing the excitability of magnocellular endocrine neurons, nicotinic agonists can influence the release of vasopressin and oxytocin from the neurohypophysis. By contrast, they appear to have no direct effects on paraventricular parvocellular neurons.

This work was supported by the Swiss National Science Foundation.

ALTERED ACTIVITY OF CHOLINERGIC ENZYMES IN MUSCLES AND BRAIN OF THE OBESE-DIABETIC (OB/OB) MOUSE

M.C. Lintern, L. Cooke, H. Scriven, M.E. Smith

Department of Physiology, Medical School, University of Birmingham, UK

The activity of acetylcholinesterase molecular forms was determined in the diaphragm, extensor digitorum longus (EDL) and soleus muscles, and the brain of obese diabetic (ob/ob) mice of different ages and their lean littermates. Three peaks of activity, representing the G1, G4 and A12 molecular forms were separated in the muscles and two peaks, representing the G1 and G4 forms in the brain. In the diaphragm, at 10-12 weeks of age, the activity of all molecular forms of the enzymes was significantly higher in the diabetic mouse than in controls. In the EDL the activity of only the G4 form was significantly higher than in the controls. In the soleus there were no significant differences between the two groups of mice. In the brain tissue the activity of both the G1 and G4 molecular forms was significantly lower in the obese mice than in the lean mice. Neuropathy is often present in non-insulin dependent (type 2) diabetes and defects in glutamatergic transmission have been reported. However this is the first report of changes in the cholinergic system. The importance of these findings in diabetes is as yet unclear but they may be relevant to the presence of cognitive defects in the condition.

UNDERSTANDING THE DUAL MODE OF ACTION OF REMINYL(R) USING A VIRTUAL SYNAPTIC CLEFT

M. Lazarewicz, A. Spiros, L. Finkel, R. Carr, H. Geerts

In Silico Biosciences, Philadelphia, PA, USA

Objective: Galantamine (Reminyl(R); GAL) is both an acetylcholinesterase (AChE) inhibitor and an allosteric modulator at nicotinic acetylcholine (ACh) receptors (nAChR). As these two cholinergic effects are interdependent, the overall effect of GAL on transmission is a complex function of the synaptic concentration of GAL. We have addressed this problem using a computer simulation based on mathematical descriptions of known physicochemical interactions. Methods: The virtual synaptic cleft is a computer model incorporating the latest neuroanatomical and neurophysiological data on the cholinergic synapse. It includes a full description of the kinetic transitions associated with both the $\alpha 4\beta 2$ and the $\alpha 7$ nAChR, and describes the potentiating interaction between GAL and the $\alpha 4\beta 2$ nAChR. Results: Overly potent AChE inhibition can drive nicotinic receptors into desensitization, especially under phasic activation of the synapse. As a consequence, the beneficial effects of increasing ACh are reduced. By introducing neuropathology data from AD tissue, the model allows assessment of cholinergic transmission deficits, and evaluation of the effect of pure AChE inhibition and of GAL in synapses from mild versus moderate AD. The virtual synaptic cleft also allows focus on the interaction between cholinergic and dopaminergic neurotransmission (modelled after the neurophysiology of the striatum); simulations show a unique effect of GAL on dopamine levels. Conclusion: This computer simulation allows investigation of the molecular interactions of complex neurological systems, where different subsystems are active simultaneously. The web-based nature of this application ensures maximal flexibility for worldwide use and increases its usefulness as a knowledge reference database.

EFFECT OF PYRIDOSTIGMINE ADMINISTRATION ON ACETYLCHOLINESTERASE AND CHOLINEACETYLTRANSFERASE ACTIVITY IN THE GUINEA-PIG STRIATUM AND CEREBELLUM

M.E. Smith¹, M.C. Lintern¹, C.J. Brewer¹, J.R. Wetherell²

¹Department of Physiology, Medical School, University of Birmingham,

²DSTL, Chemical and Biological Sciences, Porton Down, Salisbury, UK

Pyridostigmine bromide or saline (controls) was administered continuously for 6 days in guinea pigs, via osmotic pumps. The activities of G4 functional acetylcholinesterase (AChE) and cholineacetyltransferase (ChAT) were measured in striatum and cerebellum, (regions of high and low levels of acetylcholine respectively). Measurements were made on day 6, and at 1, 7, or 13 days after the treatment. In the pyridostigmine-treated animals at 6 days the activity of G4 AChE in the striatum was slightly higher than in controls, but the ChAT activity was approximately twice that in controls. At one day after the treatment ChAT activity and AChE activity were both lower than in controls. Thereafter both enzymes gradually increased up to 13 days after the treatment. A different pattern was seen in the cerebellum. In the pyridostigmine-treated animals at 6 days AChE activity was lower than in controls, but on day 7 it was higher. There was no significant change in ChAT activity at any time point examined. In the case of AChE the activity was measured at a time when the inhibitory action of the drug would long have worn off. Therefore the changes in activity of both enzymes were probably due to altered expression of the enzymes. Pyridostigmine does not readily cross the blood-brain barrier. Therefore the changes in the enzyme activities in the brain tissues could be secondary to a peripheral action of the drug. Alternatively the drug or the anaesthetic used could have permeabilised the barrier.

This work was supported by dstl. C. British Crown copyright 2001/dstl. Reproduced with permission of Her Britannic Majesty's Stationery Office.

NICOTINIC BETA4 RECEPTOR MEDIATED ACETYLCHOLINE RELEASE FROM RAT INTERPEDUNCULAR NUCLEUS

F.A. Jones, L.R. Johnson, N. Evans, S. Bose, P.J. Craig, S.G. Volsen, I.A. Pullar

Eli Lilly & Company Ltd., ErlWood Manor, Windlesham, Surrey, UK

Previous studies (Grady et al., 2001) have shown that acetylcholine (ACh) release from mouse interpeduncular nucleus (IPN) is predominantly mediated by nicotinic receptors containing the beta4 subunit. We studied the effects of different nicotinic agonists on ACh release from rat IPN. In addition we used immunolocalisation techniques to determine the distribution of the beta4 subunit within the rat brain. ACh release was measured from superfused synaptosomes that had been pre-labelled with [3H]-choline. Nicotine and cytosine stimulated ACh release in a dose dependent manner, which could be blocked by both mecamylamine (10 microM) and dihydro-beta-erythroidine (100 microM), but not by alpha-bungarotoxin (40nM). In the immunolocalisation studies a rabbit polyclonal antibody raised against a peptide mapping the intracellular loop of the rat beta4 receptor was evaluated, and its monospecificity confirmed using a range of techniques. Beta4 immunoreactivity was demonstrated in a number of brain regions but was particularly prominent in the IPN and medial habenula. The data indicates that the beta4 subunit is present in the IPN of the rat and that nicotinic receptors containing this subunit are modulating ACh release in this area of the brain.

Reference: Grady S.R., Meinerz N.M., Cao J., Reynolds A.M., Picciotto M.R., Changeux J.P., McIntosh J.M., Marks M.J., Collins A.C. Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum. 2001. *Journal of Neurochemistry*, 76, 258-268.

BEYOND THE USUAL SUSPECTS. A CHOLINERGIC ROUTE FOR PANIC ATTACKS

M. Battaglia^{1,2}, A. Ogliari¹, C. Maffei¹

¹*San Raffaele University, Milan*, ²*E. Medea Scientific Institute, Bosisio, Italy*

For unknown reasons and through poorly understood mechanisms, people at risk for panic attacks are hypersensitive to suffocative stimuli and experience hyperventilation and anxiety after exposure to heightened concentrations of carbon dioxide. Similarly to the physiological reflex response to hypercapnia in animal and man the anxious response to carbon dioxide in people with panic disorder is at least partially controlled by the central muscarinic receptors (Battaglia et al., 2001). A falsifiable hypothesis (Battaglia, 2002) is offered here that some modifications of the cholinergic functions could underlie human individual differences in carbon dioxide sensitivity and proneness to experience panic attacks. The hypothesis is based upon experimental evidence (Kaufer et al., 1998) that stressful and potentially harmful stimuli prime relatively long lasting changes in cholinergic genes expression and cholinergic receptors' regulation. The adaptive sequels of these modifications include protection of the brain from overstimulation, and, at the level of the corticolimbic circuitries, promotion of passive avoidance and learning after stress. The extension of the same modifications to the cholinergic receptors involved in chemoception, however, could lower the threshold for reaction to suffocative stimuli, including carbon dioxide. The exaggerated sensitivity to carbon dioxide observed in humans suffering from panic attacks could then be thought of as an evolutionary cost of the involvement of the cholinergic system in shaping otherwise adaptive responses to stress and threatening stimuli.

CALCIUM CONDUCTANCE AND CHOLINE SENSITIVITY OF SLOW CHANNEL SYNDROME ACETYLCHOLINE RECEPTOR MUTANTS

Ian Spreadbury, Richard Webster, David Beeson, Angela Vincent

Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

The slow channel myasthenic syndrome is caused by mutations in genes for the acetylcholine receptor (AChR), which lead to prolonged AChR openings. It is thought that the myasthenic weakness is due to an "endplate myopathy", resulting from excess calcium entry during the prolonged ACh-induced bursts; and also from choline-induced openings that occur with much greater frequency in mutant channels than in wild type. However, the calcium conductance and choline-induced calcium permeability of mutant and wild-type nAChRs have not been compared.

Single channel conductance and calcium permeability of epsilon L221F and wild type nAChRs were studied using outside-out patches from transiently transfected HEK 293 cells. As reported for other mutants, the mutant channels were 20 fold more sensitive to cholinergic agonists. However, the voltage/current relationships at different calcium concentrations were not different between mutant and wildtype, indicating similar calcium permeability. Using ratiometric recordings of Fura-2 AM-loaded cells, internal calcium changes with 5 millimolar choline (plus 1 millimolar atropine) were larger than those with 25 micromolar nicotine, but this difference was not greater in mutant than wildtype. This result does not confirm the marked difference in choline sensitivity between wildtype and mutant AChR channels reported by Zhou et al (PNAS 1996).

SELECTIVE HISTOCHEMICAL STAINING OF PERINEURONAL ACETYLCHOLINESTERASE (AChE) IN THE LIVING ENTERIC NERVOUS SYSTEM (ENS) OF RAT AND GUINEA-PIG

S. Tsuji, R. Nakatomi, H. Tsuchiya, I. Motelica-Heino, K. Hirai, Y. Katayama, K. Ishii, T. Hashikawa

Department of Cytology, Institute of Neuroscience, Pierre et Marie Curie University, Paris, France; Laboratory for Neural Architecture, Brain Science Institute, RIKEN, Wako, Saitama, Japan; Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Chiyodaku, Tokyo, Japan

AChE activity was localized only in perineuronal space of the ENS, when they were stained alive. Absence of staining of intracellular AChE was due to impermeability of the living membrane to the histochemical medium. The rat ENS was stained by means of intracardiac perfusion of modified Karnovsky's histochemical medium. After fixation and cleavage of the intestinal layers, initial weak histochemical reaction was intensified by diaminobenzidine. In case of the guinea-pig ENS (protected with a blood-brain barrier-like structure), fresh longitudinal muscle-myenteric plexus preparations were immersed in the histochemical medium, showing intense staining without diaminobenzidine intensification. The cleaved face of preparations of the rat and guinea-pig was adequate for SEM observation. A high contrast image of histochemical precipitates (cupric ferricyanide) was obtained by the backscattered electron imaging (BSEI). The Cu and Fe of the precipitates were detected by energy dispersive X-ray analysis (EDXA), and elementary X-ray mapping of Cu and Fe on the whole specimens provided images superposable to those of the BSEI, or even finer than those of the BSEI. TEM observation confirmed fine perineuronal localization of the AChE activity. It is plausible that the perineuronal AChE plays a role for hydrolysis of ambient ACh and regulates extra-synaptic communication among neurons.

NICOTINIC ACETYLCHOLINE RECEPTOR $\alpha 5$ SUBUNITS MODULATE OXOTREMORINE-INDUCED SALIVATION AND TREMOR

Ningshan Wang¹, Avi Orr-Urtreger², Joab Chapman^{1,3},
Ruth Rabinowitz¹, Amos D. Korczyn^{1,3}

¹Department of Physiology and Pharmacology, Sackler Medical School, Tel Aviv University, ²Genetic Institute and Department of Pediatrics and ³Neurology, Tel Aviv Sourasky Medical Center and the Sieratzki Chair of Neurology, Tel Aviv University, Ramat Aviv, Israel

Objective: To determine function role of neuronal nicotinic acetylcholine receptor (nAChR) $\alpha 5$ subunits in modulating the effects of oxotremorine (OXO) on autonomic functions and tremor.

Background: In 12 distinct nAChR subunits ($\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$), $\alpha 5$ subunits are expressed throughout central and autonomic nervous systems and have unique properties in modulation of ACh transmission.

Design/Methods: OXO-induced autonomic functions and tremor were measured in mice lacking $\alpha 5$ subunits ($\alpha 5$ -/-) and wild-type (WT) control mice. OXO was injected subcutaneously in gradually increasing doses: 0.01, 0.03, 0.1, 0.3 and 0.5 mg/kg in 40 min intervals. The effects of OXO were tested on awake mice (hypothermia, tremor and salivation) and on mice under anesthesia (bradycardia, defecation and salivation).

Results: Injection of OXO produced dose-dependent whole body tremor, salivation, and hypothermia with maximal responses obtained at a dose of 0.3 mg/kg, in both $\alpha 5$ -/- (n=4) and WT control (n=7) awake mice. However, $\alpha 5$ -/- mice showed significantly greater intensities of salivation and tremor responses to low OXO dose. For example at 0.03 mg/kg $\alpha 5$ -/- mice reached near maximal responses (about 90%), while WT mice reached only 40 and 50% of maximal salivary ($F_{1,9}=17.2$) and tremor ($F_{1,9}=11.28$) responses, respectively ($p<0.01$, one way ANOVA, Dunnett multiple comparison). The hypothermia, bradycardic and defecating effects induced by OXO were of similar magnitudes in the two mouse strains.

Conclusion: The increased OXO effects in $\alpha 5$ -/- mice might be due to elimination of $\alpha 5$ inhibitory effects, and support the participation of $\alpha 5$ subunits in cholinergic transmission in autonomic ganglia.

Supported by the Sieratzki Chair of Neurology, Tel Aviv University and the Miriam Turjanski de Gold and Dr. Roberto Gold Fund for Neurological Research.

DEFICIENCY OF $\beta 4$ NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS CAUSES AUTONOMIC CARDIAC AND INTESTINAL DYSFUNCTIONS

Ningshan Wang¹, Avi Orr-Urtreger², Joab Chapman^{1,3}, Ruth Rabinowitz¹,
Rachel Nachman¹, Amos D. Korczyn^{1,3}

¹Department of Physiology and Pharmacology, Sackler Medical School, Tel Aviv University, ²Genetic Institute and Department of Pediatrics and ³Neurology, Tel Aviv Sourasky Medical Center and the Sieratzki Chair of Neurology, Tel Aviv University, Ramat Aviv, Israel

Objective: To investigate the physiological and pharmacological functions of $\beta 4$ neuronal nicotinic acetylcholine receptors (nAChRs) subunits in autonomic nervous system (ANS).

Background: nAChR $\beta 4$ subunits are expressed in ANS and influence many properties of physiology and pharmacology.

Design/Methods: Autonomic functions were measured in knockout mice lacking nAChR subunit $\beta 4$ ($\beta 4$ -/-) and wild-type (WT) mice.

Results: $\beta 4$ -/- mice grew to normal size without showing any obvious physical, neurological or autonomic deficits. There was no difference between $\beta 4$ -/- and WT mice on the rectal temperature changes during exposure to cold stress (6 °C) and following 30 mg/kg morphine, as well as on pupillary size changes following morphine. Heart rate at rest, stressed by cage shaking, during exposure to cold stress or anaesthetized was not significantly different between the $\beta 4$ -/- and WT mice. During high frequency of vagal stimulation, all the WT mice, but none of the $\beta 4$ -/- mice developed cardiac arrest. Deficiency of $\beta 4$ subunits strikingly increased the sensitivity to a low dose of C_6 (3 mg/kg). A greatly reduced ileal contractile responses to nicotinic agonists cytosine, dimethylphenylpiperazinium iodide and nicotine (10 mg/kg each), and epibatidine (0.1 mg/kg) were seen in $\beta 4$ -/- mice.

Conclusion: $\beta 4$ subunits are important components in the ANS. Deficiency of $\beta 4$ subunits altered ion channel properties, conductance and sensitivity and affinity of receptors to agonists and antagonists leading to reduction of ganglionic transmission to end-organs.

Supported by the Sieratzki Chair of Neurology, Tel Aviv University and the Miriam Turjanski de Gold and Dr. Roberto Gold Fund for Neurological Research.

AUTONOMIC FUNCTION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS $\alpha 5$ SUBUNITS

Ningshan Wang¹, Avi Orr-Urtreger², Joab Chapman^{1,3}, Ruth Rabinowitz¹,
Rachel Nachman¹, Amos D. Korczyn^{1,3}

¹Department of Physiology and Pharmacology, Sackler Medical School, Tel Aviv University, ²Genetic Institute and Department of Pediatrics and ³Neurology, Tel Aviv Sourasky Medical Center and the Sieratzki Chair of Neurology, Tel Aviv University, Ramat Aviv, Israel

Objective: To determine the function role of neuronal nicotinic acetylcholine receptor (nAChR) $\alpha 5$ subunits in autonomic ganglia.

Background: In 12 distinct nAChR subunits ($\alpha 2$ - $\alpha 10$, $\beta 2$ - $\beta 4$), $\alpha 5$ subunits have unique properties in their sequences and their combinations with other subunits. The functional role of $\alpha 5$ subunits in autonomic ganglia are not well-known.

Design/Methods: Autonomic functions were measured in mice lacking $\alpha 5$ subunits ($\alpha 5$ -/-) and wild-type (WT) control mice to characterize the properties of $\alpha 5$ subunits under physiological conditions and following pharmacological interventions.

Results: All $\alpha 5$ -/- mice grew to normal size showing no obvious physical, neurological or autonomic deficit. **Similarities between $\alpha 5$ -/- and WT mice:** The rectal temperatures in ambient temperatures of 21 °C and cold exposure (6 °C); Pupil size at rest and following morphine (30mg/kg); Heart rate at rest, under anesthesia and stressed by cage shaking and cold exposure. Deficiency of $\alpha 5$ subunits strikingly increased the sensitivity to a low dose of hexamethonium (C_6) leading to a nearly complete blockade of bradycardia in response to vagal stimulation as well as elimination of rebound post vagal-stimulation tachycardia. Such a dose of C_6 only slightly depressed the effects of vagal stimulation in control mice. An impairment of cardiac parasympathetic ganglionic transmission was observed during high frequency cervical vagal stimulation. Another strikingly difference was that deficiency of $\alpha 5$ subunits significantly increased ileal contractile responses to cytosine and epibatidine (but not to dimethylphenylpiperazinium iodide and nicotine).

Conclusion: $\alpha 5$ nAChR subunits are normally present in ANS ganglia, probably modulating postsynaptic nAChR channels responses to endogenous ACh and regulating responses to ganglion drugs in receptor complexes, such effects of $\alpha 5$ subunits may lower the safety factor in transmission systems.

$\alpha 7$ ACETYLCHOLINE RECEPTOR IN SCHIZOPHRENIA: DECREASED mRNA LEVELS IN PERIPHERAL BLOOD LYMPHOCYTES

Orly Perl¹, Tal Ilani¹, Rael D. Strous², Sara Fuchs¹

¹Department of Immunology, The Weizmann Institute of Science, Rehovot, and ²Beer Yaacov Mental Health Center, Beer Yaacov, Israel

Central cholinergic systems are known to control basic functions of the brain. Recent studies have suggested that $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ AChR) may be associated with some aspects of schizophrenia. Reduced amounts of brain $\alpha 7$ AChR in schizophrenics as compared with healthy controls has been reported. In search for peripheral biological markers for schizophrenia that may enable measurable and rapid diagnosis of this disorder we have investigated $\alpha 7$ mRNA levels in peripheral blood lymphocytes (PBLs) of schizophrenic patients and healthy controls. Blood samples were collected from 34 medicated and unmedicated (drug naïve) schizophrenic patients, and from 21 healthy smokers and nonsmokers. RNA was prepared from isolated lymphocytes and its amount and quality determined. PCR products, specific for human $\alpha 7$ AChR, were quantified by densitometry using Scion image analysis software. A significant decrease (20-95%) of $\alpha 7$ mRNA levels in PBLs of schizophrenic patients has been observed, compared with controls. The decrease in $\alpha 7$ mRNA levels was not a result of medication, since unmedicated patients showed the same range of reduction as hospitalised schizophrenic patients. In addition, we have ruled out the possibility that the observed decrease in $\alpha 7$ mRNA levels resulted from nicotine consumption by smoking, as healthy smokers exhibited the same levels of $\alpha 7$ mRNA as nonsmokers. We propose that $\alpha 7$ AChR mRNA may serve as a peripheral marker for schizophrenia. Moreover, if the observed changes in $\alpha 7$ mRNA levels in PBLs indeed reflect the state of this receptor in the brain, our findings support the assumption that a deficit in $\alpha 7$ AChR is involved in the pathophysiology of schizophrenia.

**USE OF THE MORPHING GRAPHICS TECHNIQUE TO
VISUALIZE CONFORMATIONAL DIFFERENCES BETWEEN
ACHES FROM DIFFERENT SPECIES AND INHIBITOR-INDUCED
CONFORMATIONAL CHANGES**

T. Zeev-Ben-Mordehai¹, I. Silman², J. L. Sussman¹

*Departments of Structural Biology¹ and Neurobiology²,
Weizmann Institute of Science, Rehovot, Israel.*

There are currently more than 25 AChE structures deposited in the Protein Data Bank, from four different species and/or complexed or conjugated with a repertoire of ligands. A method for sorting and characterizing differences between these structures is presented.

Pairs of AChE structures were aligned using LSQMAN, and rmsd values were calculated. Intermediate models between the two structures were produced by LSQMAN, in Cartesian space, by taking the initial and final coordinates, and interpolating the predicted intermediate coordinates. The intermediate models were then collated into a single QuickTime movie file easily viewable on most computers.

This morphing approach highlighted a conformational difference in loop 319-324 (hAChE numbering) between hAChE and TcAChE earlier reported by Kryger *et al* (*Acta Cryst.* [2000] **D56**:1385). A similar conformational difference in the same loop between DmAChE and TcAChE was pinpointed utilizing the novel procedure.

A series of movies was compiled, comparing native TcAChE with its complexes and conjugates with a number of inhibitors. These reveal significant inhibitor-induced conformational changes at the top of the active-site gorge. A major conformational change was visualized for the conjugate of TcAChE with diisopropylphosphorofluoridate (DFP).

The simple morphing technique developed thus provides a valuable tool for locating and assessing conformational differences between closely related protein structures.

XIth INTERNATIONAL
SYMPOSIUM ON
**CHOLINERGIC MECHANISMS-
FUNCTION AND DYSFUNCTION**
&
2nd MISRAHI SYMPOSIUM ON
NEUROBIOLOGY



INDEX



	Program Page	Abstract Page
A		
Adams, D.J.	22	29
Adams, M.E.	12	4
Adani, R.	18	19
Ahdut, R.	13	6
Albuquerque, E.X.	16	13
Alewood, P.F.	22	29
Alkondon, M.	16	13
Allen, T.B.	31	55
Alvarez, A.	15	13
Amit, T.	18	18
Amitai, G.	18	19
Andrews, J.	21	23
Andrews, M.C.	25	38
Anglister, J.	11	3
Anglister, L.	12,21	5,25
Angus, L.M.	12	5
Antil-Delbeke, S.	11	2
Antollini, S.	11	2
Antonin, W.	13	20
Ariel, N.	14,24	8,9,34
Arpagaus, M.	12	6
Ashani, Y.	14	10
Auld, V.J.	21	24
Aviv, E.	17	11
Avraham, Y.	30	51
Azeeva, E.A.	22	27
B		
Badet, B.	25	36
Baier, J.	11	2
Baker, S.R.	28	48
Balass, M.	11	2
Bancila, V.	26,31	41,56
Baptista, G.	22	30
Barak, D.	14	8,9,34
Barak, R.	24	34
Bar-Am, O.	18	18
Bar-Her, N.	16	15
Barnard, E.A.	12	4
Bar-On, P.	14, 24	8,34
Barrantes, F.J.	11	2
Barril, X.	24	36
Bartolucci, C.	25	37
Bass, C.G.	25	38
Battaglia, M.	31	58
Bauer, B.	17	10
Beattie, R.E.	22	29
Bednar, I.	27	44
Bee, M.S.	18	19
Beeson, D.	28,31	46,58
Behra, M.	22	28
Bekpinar, S.	30	52
Belanger, G.	12	5
Bell, E.	22	29
Ben Chaim, Y.	27	43

	Program Page	Abstract Page
Ben Shushan, D.	30	51
Beni, S.M.	16	15
Berkovic, S.F.	21	26
Berman, H.A.	24,25	33,39
Bermudez, I.	23	31
Bernard, V.	28	49
Berry, E.M.	30	51
Berse, B.	13, 27	17,42
Bertrand, C.	22	28
Bertrand, D.C.	11,21,22,31	3,24,26,27,29,57
Bertrand, S.	21,22	24,26,29
Bezakova, G.	12	3
Birikh, K.R.	27	45
Bixel, G.M.	11	2
Blank, P.	13	6
Blanton, M.	11	2
Bledi, Y.	12	7
Bloc, A.	25,26,31	37,41,56
Bloch, B.	28	49
Blum, B.	30	50
Blusztajn, J.K.	13, 27	17,42
Bogoch, Y.	12	7
Bon, C.	25	37
Bon, S.	21	25
Boneva, N.		45
Bonini, I.	11	2
Bons, N.	16	15
Boot, J.R.	28	48
Bose, S.	22,31	29,58
Boss, A.	30	53
Botti, S.	21	24
Bourne, Y.	22,25,26	26,39,41
Boyd, A.	25	36
Brana, C.	28	49
Brandeis, R.	16	15
Brejč, K.	11	1
Brenner, T.	27	45
Brewer, C.J.	31	57
Brimijoin, S.	15	13
Broad, L.M.	16,22	14,29
Brochier, L.	25	38
Bronfman, M.	15	13
Broomfield, C.A.	27	43
Brovtsyna, N.B.	24	32
Brownlow, S.	28	46
Brydson, M.	28	46
Bueters, T.J.H.	14,27	10,42
Bunc, M.	25	39
Bymaster, F.P.	17,18,28	16,19,47
C		
Caccin, P.	27	45
Cambi, F.	21	23
Camp, S.	13	6
Camps, P.	24	36
Cangioli, J.	17	16
Capsoni, S.	31	55,56

	Program Page	Abstract Page
Carlier, P.R.	24	35
Carr, R.	31	57
Cartaud, A.	22	28
Cartaud, J.	22	28,29
Casu, M.A.	17	16
Cattaneo, A.	31	55,56
Changeux, J.P.	11,22	1,2,28,29,30
Chapman, J.	32	59
Chapman, P.F.	30	50
Charpantier, E.	22	27
Chatonnet, A.	22	28
Che, C.	11	3
Cheah, L.S.	22	27
Chen, C.P.L-H.	16	15
Cheng, A.W.M.	21,23	25,31
Chittlaru, T.	14,27,28	10,44,49
Choi, R.C.Y.	21,23	25,31
Clark, M.T.	14	10
Clarkson, E.D.	14	33
Cohen, O.	27,28	44,49
Combes, D.	12	6
Comoletti, D.	22	26
Cooke, L.	31	57
Coorsen, J.	13	6
Cordeiro, M.	26	41
Correia-de-Sa, P.	25,28	38,48
Corringer, P.J.	11	2
Cottage, E.L.A.	29	50
Cottingham, M.G.	31	56
Cousin, X.	22	28
Craig, P.J.	22,31	29,58
Crawford, N.	22	28
Crayton, J.W.	30	53
Crne-Finderle, N.	17	15
Cronin, T.	14	33
Croxen, R.	28	46
Cuello, A.C.	17	16
Cummings, J.L.	31	55
Curtis, C.A.M.	18	19
D		
Danhof, M.	14	10
Dascal, N.	27	43
Davidson, E.	21	23
Davies, B.M.	28	47
De Ferrari, G.V.	15	13
De Koninck, Y.	17	16
De los Santos, B.	11	2
De Vente, J.	30	52
Dell, C.P.	31	54
Deng, C.	18	19
Depoboylu, C.	12	13
Dergousova, N.I.	22	27
Deschenes, J.	12	5
Devillers-Thiery, A.	22	29
Devonshire, A.L.	23,26	31,40
Dgany, O.	24	34

	Program Page	Abstract Page
Dinov, I.D.	31	55
Dishon, S.	27	44
Dobson, D.R.	31	54
Doctor, B.P.	14,24,25,30	10,33,34,38,52,53
Dolezal, V.	25	37
Dolly, J.O.	13	7
Domenici, L.	31	56
Drews, U.	16,30	53
Ducancel, F.	21	26
Dunant, Y.	13,25,26,31	7,37,41,56
Dunbar, S.	23	31
Durrant, A.R.	21	25
Dusitsin, N.	27	42
Duttaroy, A.	18	19
Duysen, E.G.	28	46,47,48
Dvir, H.	14, 21,24	8,25,35,36
E		
Eberling, L.	28	46
Eiden, L.E.	12,28	13,47
El-Fakahany, E.	27	43
Elhanani, E.	24	34
Encinar, J.A.	22	29
Engel, A.G.	15	11
Enz, A.	18,24	34
Eroglu, L.	30	51
Esiri, M.	16	15
Evans, N.M.	22, 31	29,58
Evron, T.	27	45
Ezra, Y.	16	15
F		
Falk-Vairant, J.	25	37
Farchi, N.	28	45
Faria, M.	25	38
Fasshauer, D.	13	20
Favre, I.	21	26
Feaster, S.R.	14	33
Fedon, Y.	12	6
Felder, C.C.	17,18,28	16,19,47
Felix, J.	31	55
Fels, G.	26	40
Felthouse, C.	16	14
Fendyur, A.	13	6
Fernandez, A.M.	22	29
Finkel, L.	31	57
Finlayson, K.	22	28
Fisher, A.	16	15
Flynn, R.	22	26
Follettie, M.T.	13	17
Folly, E.A.	22	29
Fontecilla-Camps, J-C.	14,25	9,38
Foran, P.G.P.	13	7
Fournier, D.	25	38
Francis, P.T.	16,30	15,50
Fridkin, M.	11	2
Friedman, A.	17,30	11,54
Froehner, S.C.	12	4

	Program Page	Abstract Page
Froment, M-T.	26	40
Fry, D.	28	48
Fu, Y.	30	54
Fuchs, S.	11,15,32	2,12,59
Fuhrer, C.	28	49
G		
Gabel, F.	25	38
Gaillard, C.	11	2
Gallegos, M.C.	11	2
Gannon, K.S.	28	47
Gao, Y.	30	54
Garbay, C.	21	25
Garbus, I.	11	2
Garcia, G.E.	27	43
Garcia-Lopez, M.	16	
Garrido, J.L.	15	13
Geerts, H.	31	57
Giacobini, E.	18	18
Giannotta, S.	31	55
Gil, Z.	15	12
Giles, K.	14	8
Gilquin, B.	11	2
Ginzberg, D.	27	44
Giovannini, M.G.	17	16
Glick, D.	12	12
Goeldner, M.	11,21	3,24
Goerdes, M.	12	13
Golan, H.	17	11
Golicnik, M.	25	36,40
Gomez, J.	18	19
Gonzalez-Ros, J.M.	13,22	29
Gopalakrishnakone, P.	22	27
Gordon, R.K.	14,30	33,53
Gordon, T.P.	15	12
Grailhe, R.	22	28
Grantham, C.	16,21	14,23
Greenberger, V.	17	17
Greenblatt, H.M.	14,24,25	8,35,36
Groen, B.	14	10
Groot-Kormelink, P.	21	23
Grubic, Z.	12,21,22	4,23,30
Gruener, R.	27	43
Grutter, T.	11,21,22	3,24,30
Guan, Z-Z.	31	56
Guillou, C.	25	36
Gunduz, A.T.	14	33
Gunning, R.V.	25,29	39,50
Gupta, A.	26	41
Gupta, R.C.	24,26,30	33,41,51
Gur, D.	24	32
Gwee, M.C.E.	22	27
Haberberger, R.	21,28,30	24,46,54
Hale, S.L.	30	52
Halevi, S.	15	14
Hammond, P.	15	13

	Program Page	Abstract Page
Hamra, Y.	27	45
Han, Y-F.	24,30	35,54
Hao, S.	30	51
Harel, M.	11,14,21,24	2,8,25,34,36
Haring, R.	16	15
Harmelin, A.	15	12
Hart, J.	28	48
Hasegawa, H.	22	26
Hashikawa, T.	32	58
Haslam, S.	28	46
Heiss, W-D.	17	10
Hellstrom-Lindahl, E.L.	31	56
Henchman, R.H.	24,36	35,41
Herholz, K.	17	10
Hersh, L.B.	12,29	8,50
Heslop, K.E.	30	50
Hinrichs, S.	28	48
Hirai, K.	32	58
Ho, W-L.	30	54
Hochner, B.	28	45
Hoffman, R.	22	26
Hogg, R.C.	22	29
Hollinshead, M.S.	31	56
Houlihan, L.M.	23	31
Hrabovska, A.	28	46
Huang, Y.	23	31
Hucho, F.	11	2
Hulme, E.C.	18	19
I		
Ijzerman, A.P.	14	10
Ilani, T.	32	59
Im, S-H.	15	12
Inestrosa, N.C.	15	13
Ishii, K.	32	58
Israel, M.	13,25	7,37
Israeli, J.	30	50
Itier, V.	11	3
Iyengar, S.	28	48
J		
Jahn, R.	13	20
Jakubik, J.	27	43
Jasmin, B.J.	12	5
Javed, N.	26	40
Jennings, L.	22	26
Jevsek, M.	22	30
Johnson, J.L.	24	35
Johnson, L.R.	22,31	29,58
Jones, F.A.	31	54,58
K		
Kachalsky, S.	13	6
Kaiserman, I.	13	6
Kalra, A.	28	48
Kaplan, D.	14,24,28	9,34,49
Karczmar, A.G.	18,30	53
Karlin, A.	11	1
Kasher, R.	11	2

	Program Page	Abstract Page
Kasheverov, I.E.	22	27
Katayama, Y.	32	58
Katchalski-Katzir, E.	11	2
Kaufmann, K.	24	35
Kedmi, M.	15	12
Keenan, M.	28	48
Keene, J.	16	15
Kelly, J.S.	22	28
Kessler, P.	23	30
Khoo, H.E.	22	27
Kim, E.	14	9
Kim, M-H.	29	50
Kimbell, L.M.	12	4
King, M.P.	12,21	4,23
Kirwan, M.	23	31
Klaassen, R.V.	11	1
Klein, J.	17	17
Kobayashi, S.	22	30
Koellner, G.	14	8
Komives, E.	22	26
Konopka, L.M.	30	53
Kopf, S.	17	17
Korczyn, A.D.	18,32	18,59
Korn, A.	17	11
Korotina, A.S.	22	27
Kotzyba-Hibert, F.	11,21	3,24
Kovalev, E.	27	44
Kovarik, Z.	24	33
Koyuncuoglu, H.	30	52
Krauss, M.	11	2
Kreimer, D.	14	9
Krejci, A.	18	19
Krejci, E.	15,22	5,27
Kremer, A.	21	23
Kress, M.	28	46
Kronman, C.	14,24,27,28	9,10,34,44,49
Kryger, G.	14,24	8,35
Kryukova, E.V.	22	27
Kubo, T.	22	30
Kummer, W.	21,28,30	24,46,54
L		
Lamba, D.	25	37
Landman, N.	17	17
Lansdell, S.J.	23	31
Lazar, A.	27	44
Lazar, S.	27,28	44,49
Lazarewicz, M.	31	57
Le Du, M.H.	21	26
Le Novere, N.	22	30
Leader, H.	24	34
Lee, M-H.	13	8
Lee, N.T-K.	30	54
Legay, C.	21	26
Lenz, D.E.	14	10,33
Lester, S.	15	12
Lewis, T.	26	40

	Program Page	Abstract Page
Li, B.	28	47
Li, L.	13	17
Lilienfeld, S.	16	14
Linial, M.	12	7
Linnemann, E.	26	40
Lintern, M.C.	31	57
Lips, K.S.	21,28,30	24,46,54
Lisk, G.O.	13	7
Liste, I.	28	49
Liu, W.Q.	21	25
Littauer, U.	16	
Lobo, M.G.B.	25	38
Lockridge, O.	14,25,27,28	9,38,43,46,47,48,49
Loffelholz, K.	17	17
Lohitnavy, O.	27	42
Lomo, T.L.	12	3
Lopez-Coviella, I.	13,27	17,42
Loudwig, S.	11	3
Lu, Z-L.	18	19
Lukas, R.J.	27	43
Luo, C.	24	34
Luque, F.J.	24	36
Luttmann, E.	26	40
Luyten, W.	21	23
M		
Maelicke, A.	16	13,14
Maestre-Frances, N.	16	15
Maffei, C.	31	58
Makita, R.	18	19
Malo, M.	25	37
Manese, M.	31	55
Manjunatha Kini, R.	22	27
Marchand, S.	22	28,29
Marchot, P.	14,22,25,26	26,39,41
Marcion, R.	21	24
Marcovitch, I.	16	15
Margittai, M.	13	20
Markerink-van Ittersum, M.	30	52
Mars, T.	12,21,22	4,23,30
Masson, P-Y.	14,25,26	9,37,38,40
Massoulie, J.	12,21	5,25
Masure, S.	21	23
Mateo, R.	22	29
Matsumura, T.	22	26
Matthews, K.L.	30	50
Maxwell, D.M.	14,24	10,33,34
McCammon, J.A.	24,26	35,41
McEvoy, J.P.	31	55
McKinzie, D.L.	17,28	16,47,48
McMahan, U.J.	13,15	6
McPhie, G.	16	14
Mega, M.	31	55
Meilin, S.M.	16	15
Melamed-Book, N.	13	6
Mellott, T.	13,27	17,42
Mendelson, S.	30	51

	Program Page	Abstract Page
Menez, A.	11,21,23	2,26,30
Meshulam, H.	18	19
Meunier, F.A.	13	7
Michaelson, D.M.	16	15
Miles, C.	28	48
Millar, N.S.	22,23	29,31
Millard, C.B.	14,24,27	8,9,34,43
Minic, J.	15,22	5,27
Miranda, A.F.	12,21	4,23
Mis, K.	12	23
Mitchell, S.N.	28	48
Mittaud, P.	28	49
Miyakawa, T.	18	19
Mohammed, N.	13	7
Molgo, J.	15,22	5,27
Molina, M.L.	22	29
Molles, B.	13	6
Montecucco, C.	27	45
Moon, E.K.	16	13
Moorad-Doctor, D.R.	27	43
Moore, N.M.	28	48
Moore, G.D.	25,26	38,40
Moralev, S.N.	23,24	31,32
Moss, S.	28	48
Motelica-Heino, I.	32	58
Mouro, A.	11,21	3,24
Mousavi, M.	31	56
Mulley, J.C.	21	26
Murray, T.K.	28,31	48,54
Myslivecek, J.	30	52
N		
Nachman, R.	32	59
Nachon, F.	14,25,26	9,38,40
Nakatomi, R.	32	58
Nassrallah, F.	12	5
Natan, N.	16	15
Nathanson, N.M.	28	47
Neduva, V.	14	8
Newsom-Davis, J.	28	46
Nicolas, A.	11,14,25	2,9,38
Nicolet, Y.	14	9,38
Nieuwstraten, D.	21	23
Nigam, S.V.	30	53
Nirathanan, S.	22	27
Nitsch, R.	16	
Noda, S.	30	53
Nordberg, A.	27,31	44,56
Nuntharatanapong, N.	27	42
O		
O'Connor, S.M.	31	55
Ogier, R.	31	57
Ogliari, A.	31	58
Ohno, K.	15	11
Oliveira, L.	25,28	38,48
O'Neill, M.J.	31	54,48
Ophir, G.	16	15

	Program Page	Abstract Page
Oppitz, M.	30	53
Ordentlich, A.	14, 24,28	8,9,10,34,49
Oron, L.	16	15
Orozco, M.	24	36
Orr-Urtreger, A.	15,32	12,59
P		
Paas, Y	11	
Pabst, S.	13	20
Pang, Y.-P.	24	35
Park, H.	21	23
Parnas, H.	13,27	7,43
Parnas, I.	13,27	7,43
Passani, M.B.	17	16
Pavlovsky, L.	17,30	11,54
Pazzagli, M.	17	16
Pearson, K.	16	14
Pediconi, M.F.	11	2
Pepeu, G.	17	16
Perl, O.	32	59
Perry, K.W.	17	16
Pesavento, E.	31	56
Pfeil, U.	21,28,30	24,46,54
Phalach-Yogev, M.	18	18
Phillips, H.	21	26
Pilger, C.	26	40
Pittel, Z.	16	15
Pons, S.	22	29
Porter, A.	28	47
Posayanonda, T.	27	42
Poveda, J.A.	22	29
Pregelj, P.	17,21	15,23
Prieto, M.	11	2
Prieto-Da-Silva, A.	22	30
Pullar, I.A.	31	54,58
Q		
Quintero, J.M.	12	4
R		
Rabinovitz, I.	18	19
Rabinowitz, R.	32	59
Radic, Z.	13,14,24,25	6,9,33,34,36,39
Raggenbass, M.	31	57
Rahamimoff, R.	13	6
Rasmussen, K.	17	16
Raveh, A.	13	6
Ravid, R.	31	56
Recouvreur, M.	22	28
Rees, D.M.	21	24
Rees, T.	15	13
Reiner, E.	24,25	,33,36
Renault, F.	25	37
Ribeiro Da Silva, A.	17	16
Ricciardi, A.	21	26
Rigoni, M.	27	45
Rischmueller, M.	15	12
Robertson, R.	17	16
Roccamo, A.M.	11	2

	Program Page	Abstract Page
Rochu, D.	25	37
Rodriguez, E.	11	3
Rosenberry, T.L.	24	35,36
Rosenburg, Y.	14	10
Roshchina, V.V.	27	42
Ross, M.C.	14	33
Rossetto, O.	27	45
Rossi, S.G.	12,21	4,25
Roth, E.	14	9
Rotter, A.	25	39
Rotundo, R.L.	12,21	4,25
Rozengart, E.V.	23,24	32
Rydberg, E.H.	21	24
S		
Sack, R.A.	15	12
Sailer, M.	30	53
Salpeter, E.E.	15	11
Salpeter, M.M.	21	25
Salvaterra, P.M.	13	8
Samson, A.O.	11	3
Santos, M.D.	16	13
Sattelle, D.B.	15	14
Saxena, A.	14,24	10,34
Schafer, M.K-H.	12	13
Scherf, T.	11	2,3
Schopfer, L.M.	26	40
Schriek, G.	30	53
Schuetz, B.	12,28	13,47
Schuurmans, M.	11	1
Schwaeble, W.	27	42
Schwarz, T.L.	13	8
Scriven, H.	31	57
Segal, D.	24	32
Segal, M.	17	17
Segall, Y.	14,24	8,34
Seidman, S.	27	45
Seliger, N.	27,28	44,49
Sepcic, K.	25	39
Servent, D.	11	2
Shafferman, A.	14,24,27,28	8,9,10,34,44,49
Shalitin, Y.	24	32
Shan, K-R.	31	56
Shani, L.	13	6
Sharkey, J.	22	28
Sharott, A.	28	48
Shaw, D.	28	48
Shaya, D.	21,24	26,35
Shekhar, A.	17	16
Shelef, I.	17	11
Shen, T.	24,26	35,41
Sher, E.	16,22,28	14,29,48
Shi, J.	13,25	6,36
Shibanova, E.D.	22	27
Shimojo, M.	12	8
Shin, I.	14	9
Shoham, S.	27	44,45

	Program Page	Abstract Page
Shohami, E.	16	15
Silman, I.	14,21,24,25,32	8,9,24,25,26,34,35,36,38,60
Simeon-Rudolf, V.	24,25	32,33,36
Simmons, R.	28	48
Sindhupak, R.	27	42
Sine, S.M.	15	11
Sinhaseni, P.	27	42
Sinko, G.	24,25	32,33,36
Siotto, M.C.	25	37
Siow, N.L.	21,23	25,31
Sixma, T.K.	11	1
Sketelj, J.	17,21	15,23
Sklan, E.H.	27	45
Skvorak, J.P.	14	33
Slack, B.E.	13	17
Slater, C.	28	46
Slutsky, I.	13	7
Smit, A.B.	11	1
Smith, M.E.	31	57
Smulders, C.J.G.M.	27	42
Snowden, R.	27	42
Sodhof, T.	22	26
Sod-Moriah, G.	18	19
Solomon, A.	21	24
Sonego, H.	16	15
Song, S.	13	8
Soreq, H.	12,15,27,28	12,13,44,45
Souroujon, M.C.	15	12
Specht, A.	11	3
Spiros, A.	31	57
Spreadbury, I.	31	58
Steggles, D.	31	54
Steinbusch, H.W.M.	30	52
Stetzkowski-Marden, F.	22	28
Stojan, J.	25	36,40
Stribley, J.A.	28	48
Strochlic, L.	22	28
Strohle, U.	22	28
Strous, R.D.	32	59
Stuglin, A.	24,25	32,36
Suput, D.	25	39
Suramana, T.	27	42
Sussman, J.L.	11,14 21,24,25,32	2,8,24,25,26,34,35,36,60
Suvorov, A.A.	24	32
Svedberg, M.M.	27	44
Svensson, A-L.	27	44
Szabo, M.	21	25
T		
Tai, K.	24,26	35,41
Takeda, M.	22	30
Tang, X.C.	21,29	23,49
Taylor, P.	13,14,22,24,25	6,9,26,33,34,36,39
Teixeira, F.	23	30
Thakur, S.S.	24	33
Thal, C.	25	36
Thies, R.S.	13	17

	Program Page	Abstract Page
Timoteo, M.A.	25,28	38,48
Toga, A.W.	31	55
Toker, L.	14	8
Tomkins, O.	17	11
Tonkopp, V.D.	25,31	39,55
Tortella, F.	30	52
Tour, O.	27	43
Toutant, J-P.	12	6
Tree, B.	28	48
Treinin, M.	15	14
Tribollet, E.	31	57
Tricklebank, M.D.		48
Tsetlin, V.I.	16,22	27
Tsim, K.W.K.	12,21,22,23,30	4,25,27,31,54
Tsuchiya, H.	32	58
Tsuji, S.	13,26,32	41,58
Tucek, S.	18,25,27,30	19,37,43,52
Turk, T.	25	39
Turrini, P.	17	16
Tze-Kin, N.		54
U		
Unlucerci, Y.	30	52
Utkin, Y.N.	22	27
V		
Van der Helm, L.	21	23
van der Oost, J.	11	1
Van der Speak, P.	21	23
van Dijk, W.J.	11	1
van Helden, H.P.M.	14	10
Van Staveren, W.C.G.	30	52
Vandenberk, I.	21	23
Vaux, D.J.T.	31	56
Ved, H.S.	30	52,53
Velan, B.	14,24,27,28	8,9,10,34,44,49
Vidmar, A.	25	39
Vijverberg, H.P.M.	27	42
Vincent, A.	15,28,31	11,46,58
Viner, R.C.	26	40
Volsen, S.G.	22,31	29,58
Voytenko, S.	27	43
Vonesch, J-L.	22,27	28,43
W		
Wan, D.C.C.	21,22,23	25,27,31
Wang, N.	32	59
Wang, R-H.	18	18
Waterman, S.A.	15	12
Webster, R.G.	28,31	46,58
Weihe, E.	12,28	13,47
Weik, M.	25	38
Weiner, L.	14	9
Weinstock, M.	18	18
Weisenbach, S.	17	10
Wenz, J.	11	2
Wess, J.	18,28	19,47
Wetherell, J.R.	31	57
Whittaker, V.P.	11	1

	Program Page	Abstract Page
Wiegand, S.	28	46
Williams, A.	30	52
Williamson, M.S.	23,25,26	31,38,40
Willmann, R.	28	49
Windass, J.D.	23	31
Wishart, G.	28	48
Wong, D.M.	24	36
Wong, D.W.	24	35
Wong, T.P.	17	16
Woodruff-Pak, D.	16	20
Wu, D-C.	30	54
X		
Xiao, X-Q.	30	54
Y		
Yamada, M.	18	19
Yamane, T.	22	30
Yamanturk, P.	22,30	51,52
Yang, X.	22	30
Yassin, L.	15	14
Yirmiyah, R.	27	44
Yon, J.	21	23
Youdim, M.B.H.	18	18
Young, C.	28	46
Younkin, S.	15	13
Yu, J.	17	16
Yu, K.J.	21	23
Z		
Zaccai, G.	25	38
Zaninetti, M.	31	57
Zeev Ben-Mordechai, T.	21,32	24,60
Zemlickova, V.	25	37
Zhang, H.Y.	29	49
Zhang, W.	18	19
Zhang, X.	22	27
Zilberstein, L.	27	44
Zimmerberg, J.	13	6
Zundorf, G.	17	10
Zwart, R.	16,22	14,29